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(57) Abstract

The subject invention relates to the identification of a gene involved in the desaturation of polyunsaturated fatty acids at carbon 5 (i.e., "human Δ5–desaturase") and to uses thereof. In particular, human Δ5–desaturase may be utilized, for example, in the conversion of dihomo–γ – linolenic acid (DGLA) to arachidonic acid (AA) and in the conversion of 20:4n–3 to eicosapentaenoic acid (EPA). AA or polyunsaturated fatty acids produced therefrom may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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HUMAN DESATURASE GENE AND USES THEREOF

The subject application is a Continuation-In-Part of pending U.S. Patent Application Serial No. 09/227,613 filed on January 8, 1999, which is a Continuation-In-Part of pending International Application PCT/US98/07422 filed on April 10, 1998 (which designates the U.S.) which is a Continuation-In-Part of pending U.S. Patent Application Serial No. 08/833,610 filed on April 11, 1997, all of which are incorporated herein in their entirety by reference.

BACKGROUND OF THE INVENTION

Technical Field

The subject invention relates to the identification and isolation of a gene that encodes an enzyme (i.e., human Δ5-desaturase) involved in the synthesis of polyunsaturated fatty acids and to uses thereof. In particular, Δ5-desaturase catalyzes the conversion of, for example, dihomo-γ-linolenic acid (DGLA) to arachidonic acid (AA) and (n-3)-eicosatetraenoic acid (20:4n-3) to eicosapentaenoic acid (20:5n-3). The converted product may then be utilized as a substrate in the production of other polyunsaturated fatty acids (PUFAs). The product or other polyunsaturated fatty acids may be added to pharmaceutical compositions, nutritional composition, animal feeds as well as other products such as cosmetics.

Background Information

Desaturases are critical in the production of long-chain polyunsaturated fatty acids which have many important functions. For example, PUFAs are important components of the plasma membrane of a cell, where they are found in the form of phospholipids. They also serve as precursors to mammalian

prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, in an efficient manner.

A number of enzymes are involved in PUFA biosynthesis including Δ5-desaturase (see Figure 11). For example, elongase (elo) catalyzes the conversion of γ-linolenic acid (GLA) to dihomo-γ-linolenic acid (DGLA) and of stearidonic acid (18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Linoleic acid (LA, 18:2-Δ9,12 or 18:2n-6) is prodced from oleic acid (18:1-Δ9) by a Δ12-desaturase. GLA (18:3-Δ6,9,12) is produced from linoleic acid by a Δ6-desaturase.

It must be noted that animals cannot desaturate beyond the Δ9 position and therefore cannot convert oleic acid into linoleic acid. Likewise, α-linolenic acid (ALA, 18:3-Δ9,12,15) cannot be synthesized by mammals. However, α-linolenic acid can be converted to stearidonic acid (STA, 18:4-Δ6,9,12,15) by a Δ6-desaturase (see PCT publication WO 96/13591 and The Faseb Journal, Abstracts, Part I, Abstract 3093, page A532 (Experimental Biology 98, San Francisco, CA, April 18-22, 1998) see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4-Δ8,11,14,17) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4-Δ8,11,14,17) can then be converted to eicosapentaenoic acid (EPA, 20:5-Δ5,8,11,14,17) by a Δ5-desaturase, such as that of the present invention. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbon 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and carbon 15 (see PCT

publication WO 93/11245). The major polyunsaturated fatty acid of animals therefore are either derived from diet and/or from desaturation and elongation of linoleic acid or α-linolenic acid. In view of these difficulties, it is of significant interest to isolate genes involved in PUFA synthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant, or animal system which can be altered to provide production of commercial quantities of one or more PUFAs. One of the most important long chain PUFAs, noted above, is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and adrenal glands. As noted above, AA production from dihomo-γ-linolenic acid is catalyzed by a Δ5-desaturase. EPA is another important long-chain PUFA. EPA is found in fungi and also in marine oils. As noted above, EPA is produced from (n-3)-eicosatetraenoic acid and is catalyzed by a Δ5-desaturase.

In view of the above discussion, there is a definite need for the Δ5-desaturase enzyme, the gene encoding this enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFAs beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the Δ5-desaturase gene.

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

The present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence shown in SEQ ID NO:1 (Figure 12).

The isolated nucleotide sequence may be represented by SEQ ID NO:1. These sequences may encode a functionally active desaturase which utilizes a polyunsaturated fatty acid as a substrate. The sequences may be derived from a mammal such as, for example, a human.

The present invention also includes purified proteins encoded by the nucleotide sequences referred to above. Additionally, the present invention includes a purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of the purified proteins referred to directly above.

Furthermore, the present invention also encompasses a method of producing a human Δ5-desaturase. This method comprises the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 12); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the human Δ5-desaturase. The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. In particular, the prokaryotic cell may be, for example, E. coli, cyanobacteria or B. subtilis. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell (e.g., a yeast cell such as Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. or Pichia spp.).

Additionally, the present invention also encompasses a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 12) operably linked to b) a promoter. The invention also includes a host cell comprising this vector.

The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. Suitable eukaryotic cells and prokaryotic cells are as defined above.

Moreover, the present invention also includes a plant cell, plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, selected from the group consisting of AA and EPA. The invention also includes one or more plant oils or acids expressed by the above plant cell, plant or plant tissue.

Additionally, the present invention also encompasses a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Also, the invention includes a mammalian cell comprising the above vector wherein expression of the nucleotide sequence of the vector results in production of altered levels of AA or EPA when the cell is grown in a culture media comprising a fatty acid selected from the group consisting of an essential fatty acid, LA and ALA.

It should also be noted that the present invention encompasses a transgenic, non-human mammal whose genome comprises a DNA sequence encoding a human Δ5-desaturase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 12). Additionally, the present invention includes a fluid (e.g., milk) produced by the transgenic, non-human mammal wherein the fluid comprises a detectable level of at least human Δ5-desaturase.

Additionally, the present invention includes a method (i.e., "first" method) for producing a polyunsaturated fatty

acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 12); b) constructing a vector comprising the isolated nucleotide sequence; c) introducing the vector into a host cell under time and conditions sufficient for expression of the human Δ5-desaturase enzyme; and d) exposing the expressed human Δ5-desaturase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, DGLA or 20:4n-3 and the product polyunsaturated fatty acid may be, for example, AA or EPA, respectively. This method may further comprise the step of exposing the product polyunsaturated fatty acid to an elongase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid (i.e., "second" method). In this method containing the additional step (i.e., "second" method), the product polyunsaturated fatty acid may be, for example, AA or EPA, and the "another" polyunsaturated fatty acid may be adrenic acid or (n-3)-docosapentaenoic acid, respectively. The method containing the additional step may further comprise a step of exposing the another polyunsaturated fatty acid to an additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid (i.e., "third" method). The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

The present invention also encompasses a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the "first" method, another polyunsaturated fatty acid produced according to the "second" method, and the final polyunsaturated fatty acid

produced according to the "third" method. The product polyunsaturated fatty acid may be, for example, AA or EPA. The another polyunsaturated fatty acid may be, for example, adrenic acid or (n-3)-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or DHA. This nutritional composition, may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or to an animal. It may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and protein hydrolysates. Additionally, the composition may further comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

Furthermore, the present invention also includes a a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the "first" method, the another polyunsaturated fatty acid produced according to the "second" method, and the final polyunsaturated fatty acid produced according to the "third" method and 2) a pharmaceutically acceptable carrier. Again, the pharmaceutical composition may be administered to a human or to an animal. The composition may further comprise an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an

amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

Additionally, the present invention includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the first method, the another polyunsaturated fatty acid produced according to the second method and the final polyunsaturated fatty acid produced according to the third method. The product polyunsaturated fatty acid may be, for example, AA or EPA. The another polyunsaturated fatty acid may be, for example, adrenic acid or (n-3)-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or DHA.

Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the first method, the another polyunsaturated fatty acid produced according to the second method, and the final polyunsaturated fatty acid produced according to the third method.

Additionally, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition of above in an amount sufficient to effect prevention or treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 outlines the sections of the *M. alpina* Δ5- and Δ6-desaturases, the clone ID's from the LifeSeq database to which those sections had homology, and the keyword associated with the clone ID's.

Figure 2 represents the contig 2692004.

Figure 3 represents the contig 2153526.

Figure 4 represents the contig 3506132.

Figure 5 represents the contig 3854933.

Figure 6 represents the contig 2511785.

Figure 7 represents the contig 2535 generated based on contig 2511785 of Figure 6 and contig 3506132 of Figure 4.

Figure 8 represents the contig 253538a generated based on contig 2535 of Figure 7 and contig 3854933 of Figure 5.

Figure 9 represents the amino acid sequence identity between the *M. alpina* Δ5-desaturase (Ma29) and the contig 253538a.

Figure 10 represents the amino acid sequence identity between the *M. alpina* Δ6-desaturase (Ma524) and the contig 253538a.

Figure 11 represents various fatty acid biosynthesis pathways. The role of the Δ5-desaturase enzyme should be noted.

Figure 12 represents the complete nucleotide sequence of the human Δ5-desaturase gene (human Δ5).

Figure 13 represents the amino acid sequence of the human Δ5-desaturase translated from human Δ5 (see Figure 12).

Figure 14 illustrates the sequence identity between the pRAE-7 and pRAE-8 clones.

Figure 15 represents the complete putative human desaturase gene sequence from clone pRAE-7.

Figure 16 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the *M. alpina* Δ5-desaturase.

Figure 17 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the *M. alpina* Δ6-desaturase.

Figure 18 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the contig 2535.

Figure 19 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the contig 38.

Figure 20 illustrates the amino acid sequence identity between the N-terminus of clone A-1, a representative of Group 1, and the N-terminus of cytochrome b5 gene.

Figure 21 illustrates the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-1 and a portion of the GenBank sequence ac004228.

Figure 22 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone 3-5 of Group 2 and a portion of the GenBank sequence ac004228. Clone 3-5 has an ATG within a *Nco*I site, but translates four stops between the ATG and the *BamH*I site.

Figure 23 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-10 of Group 3 and a portion of the GenBank sequence ac004228. Clone A-10 has an ATG 135 bp upstream of the *BamH*I site, giving an open reading frame of 1267 bp.

Figure 24 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-16 of Group 4 and a portion of the GenBank sequence ac004228. Clone A-16 does not have an ATG; however, there is an ATG (underlined) upstream of where the sequence aligns with ac004228.

Figure 25 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-19 of Group 5 and a portion of the GenBank sequence ac004228. Clone A-19 does not have an ATG; however, this clone matches the ac004228 sequence even upstream of the *BamH*I site.

Figure 26 represents the partial nucleotide sequence of the GenBank sequence ac004228 and the representative clones from the five Groups.

Figure 27 represents the nucleotide sequence identity between the human Δ5-desaturase and contig 3381584.

Figure 28 represents the nucleotide sequence identity between the human Δ5-desaturase and contig 2153526.

Figure 29 represents the amino acid sequence identity between the human Δ5-desaturase and contig 253538a.

Figure 30 represents the amino acid sequence identity between the human Δ5-desaturase and contig 38.

Figure 31 represents the amino acid sequence identity between the *M. alpina* Δ6-desaturase (Ma524) and the human the Δ5-desaturase.

Figure 32 represents the amino acid sequence identity between the *M. alpina* Δ5-desaturase (Ma29) and the human Δ5-desaturase.

Figure 33 illustrates the human Δ5-desaturase activity of the gene in clone pRAE-28-5, compared to that in pRAE-26-1, pRAE-33, and pRAE-35, when expressed in baker's yeast.

Figure 34 illustrates the substrate specificity of the human Δ5-desaturase gene in clone pRAE-28-5, converting DGLA(20:3n-6) to AA(20:4n-6), when the gene is expressed in baker's yeast.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to the nucleotide and amino acid sequence of the Δ5-desaturase gene derived from humans. Furthermore, the subject invention also includes uses of the gene and of the enzyme encoded by this gene. For example, the gene and corresponding enzyme may be used in the production of polyunsaturated fatty acids such as, for instance, arachidonic

acid, eicosapentaenoic acid, and/or adrenic acid which may be added to pharmaceutical compositions, nutritional compositions and to other valuable products.

The Human Δ5-Desaturase Gene and Enzyme Encoded Thereby

As noted above, the enzyme encoded by the human Δ5-desaturase gene is essential in the production of highly unsaturated polyunsaturated fatty acids having a length greater than 20 carbons. The nucleotide sequence of the isolated human Δ5-desaturase gene is shown in Figure 2, and the amino acid sequence of the corresponding purified protein is shown in Figure 3.

As an example, the isolated human Δ5-desaturase gene of the present invention converts DGLA to AA or converts 20:4n-3 to EPA. Thus, neither AA nor EPA, for example, can be synthesized without the Δ5-desaturase gene (e.g., human or *M. alpina*) and enzyme encoded thereby.

It should be noted that the present invention also encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70% of the nucleotides in sequence to SEQ ID NO:1 (i.e., the nucleotide sequence of the human Δ5-desaturase gene described herein (see Figure 12)). Such sequences may be derived from non-human sources (e.g., C. elegans or mouse). Furthermore, the present invention also encompasses fragments and derivatives of the nucleotide sequence of the present invention (i.e., SEQ ID NO:1), as well as of the sequences derived from non-human sources, and having the above-described complementarity or correspondence. Functional equivalents of the above-sequences (i.e., sequences having human

Δ5-desaturase activity) are also encompassed by the present invention. The invention also includes a purified polypeptide which desaturates polyunsaturated fatty acids at the carbon 5 position and has at least about 50% amino acid similarity to the amino acid sequence of the above-noted proteins which are, in turn, encoded by the above-described nucleotide sequences.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA desaturase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence represented by SEQ ID NO:1 and shown in Figure 12. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of Tm for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating Tm have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more

important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

Production of the Human Δ5-Desaturase Enzyme

Once the gene encoding the human Δ5-desaturase enzyme has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector or construct.

The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the human Δ5-desaturase enzyme as well as any promoter which is functional in the host cell and is able to elicit expression of the human Δ5-desaturase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucoisomerase, phosphoglycerate kinase, acid phosphatase, T7, TPI, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-antigen, ovalalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell.

As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see Molecular Cloning: A Laboratory Manual, 2nd ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)). The host cell is then cultured under suitable conditions permitting expression of the desired PUFA which is then recovered and purified.

Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for example, mammalian cells, plant cells, yeast cells such as Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Lipomyces starkey, Candida spp. such as Yarrowia (Candida) lipolytica, Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora and Penicillium. Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that

autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the marker. When stable expression results from integration, the site of the construct's integration can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the human Δ5-desaturase), and ultimately the PUFA(s) of interest. More specifically, once the above-described construct is created, it may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science 278:2130-2133 (1997)). Gestation and birth are then permitted (see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFAs and thus incorporation of the gene encoding the human Δ5-desaturase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse, a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

For expression of a human Δ5-desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of a human Δ5-desaturase gene, or antisense human Δ5-desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The human Δ5-desaturase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues and/or plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained or from a different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination

region usually is selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)) or plant tissue may also be utilized as a host or host cell, respectively, for expression of the human Δ5-desaturase enzyme which may, in turn, be utilized in the production of polyunsaturated fatty acids. More specifically, desired PUFAS can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFAs, seed oil components may be manipulated through the expression of the human Δ5-desaturase gene, as well as perhaps other desaturase genes and elongase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the human Δ5-desaturase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the human Δ5-desaturase gene. The vector may also comprise one or more genes that encode other enzymes, for example, Δ4-desaturase, elongase, Δ6-desaturase, Δ12-desaturase, Δ15-desaturase, Δ17-desaturase, and/or Δ19-desaturase. The plant tissue or plant may produce the relevant substrate (e.g., DGLA, GLA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell or plant. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAs (e.g., n-6 unsaturated fatty acids such as AA, or n-3 fatty acids such as EPA or DHA) by use of a plant cell, plant tissue or plant. It should also be noted that the invention also encompasses a transgenic plant

comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector which is subsequently introduced into the host cell, are shown in Figure 11.

In view of the above, the present invention encompasses a method of producing the human Δ5-desaturase enzyme comprising the steps of: 1) isolating the nucleotide sequence of the gene encoding human Δ5-desaturase enzyme; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and conditions sufficient for the production of the desaturase enzyme.

The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the human Δ5-desaturase enzyme such that the desaturase converts the acid to a polyunsaturated fatty acid. For example, when 20:3n-6 is exposed to human Δ5-desaturase enzyme, it is converted to AA. AA may then be exposed to elongase which elongates the AA to adrenic acid (i.e., 22:4n-6). Alternatively, human Δ5-desaturase may be utilized to convert 20:4n-3 to 20:5n-3 which may be exposed to elongase and converted to (n-3)-docosapentaenoic acid. The (n-3)-docosapentaenoic acid may then be converted to DHA by use of Δ4-desaturase. Thus, human Δ5-desaturase may be used in the production of polyunsaturated fatty acids which may be used, in turn, for particular beneficial purposes.

Uses of the Human Δ5-Desaturase Gene and Enzyme Encoded Thereby

As noted above, the isolated human Δ5-desaturase gene and the desaturase enzyme encoded thereby have many uses. For example, the gene and corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, AA, adrenic acid or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g., the conversion of DGLA to AA). "Indirectly" is meant to encompass the situation where an acid is converted to another acid (i.e., a pathway intermediate) by the desaturase (e.g., DGLA to AA) and then the latter acid is converted to another acid by use of a non-desaturase enzyme (e.g., AA to adrenic acid by elongase or by use of another desaturase enzyme (e.g., AA to EPA by Δ17-desaturase.)). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the desaturase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced directly or indirectly by use of the human Δ5-desaturase gene, in accordance with the present invention, and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed search. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which

has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulas, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored

in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFAs produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight percent of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or disease states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus, fatty acids such as AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast

milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% TO 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of human Δ5-desaturase expression, as well as the expression of other desaturases and elongases, can be used to modulate PUFA levels and ratios. The PUFAs/acids produced in accordance with the present invention (e.g., AA and EPA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced using the human Δ5-desaturase gene, in accordance with the methods described herein. More specifically, such a

pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with

antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

The present invention also includes the treatment of various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the compositions of the present invention may be used to treat restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention. Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the human Δ5-desaturase enzyme, may also be used in the treatment of eczema, in the reduction of blood pressure, and in the improvement of mathematics examination scores. Additionally, the compositions of the present invention may be

used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976), reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the following non-limiting examples:

EXAMPLE I

HUMAN DESATURASE GENE SEQUENCES

As described in International Application PCT/US98/07422 (herein incorporated in its entirety by reference), the putative human desaturase gene sequences involved in long chain polyunsaturated fatty acid biosynthesis were isolated based on homology between the human cDNA sequences and *Mortierella alpina* desaturase gene sequences. The three conserved "histidine boxes" known to be conserved among membrane-bound desaturases were found. As with other membrane-bound desaturases, the final HXXHH histidine box motif was found to be QXXHH. The amino acid sequence of the putative human desaturases exhibited homology to *M. alpina* Δ5-, Δ6-, Δ9-, and Δ12-desaturases.

The *M. alpina* Δ5-desaturase and Δ6-desaturase cDNA sequences were used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, CA. The Δ5-desaturase sequence was divided into fragments: 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-446. The Δ6 desaturase sequence was divided into three fragments: 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-457. These polypeptide fragments were searched against the database using the "tblastn" algorithm. This algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands).

The polypeptide fragments 2 and 3 of *M. alpina* Δ5- and Δ6-desaturases have homologies with the CloneID sequences as outlined in Figure 1. The CloneID represents an individual sequence from the Incyte LifeSeq database. After the "tblastn" results had been reviewed, Clone Information was searched with the default settings of Stringency of >=50, and Productscore <=100 for different CloneID numbers. The Clone Information

Results displayed the information including the ClusterID, CloneID, Library, HitID, and Hit Description. When selected, the ClusterID number displayed the clone information of all the clones that belong in that ClusterID. The Assemble command assembled all of the CloneID which comprise the ClusterID. The following default setting were used for GCG (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, WI) Assembly:

Word Size: 7; Minimum Overlap: 14; Stringency: 0.8; Minimum Identity: 14; Maximum Gap: 10; Gap Weight: 8; and Length Weight: 2.

GCG Assembly Results displayed the contigs generated on the basis of sequence information within the CloneID. A contig is an alignment of DNA sequences based on areas of homology among these sequences. A new sequence (consensus sequence) was generated based on the aligned DNA sequence within a contig. The contig. containing the CloneID was identified, and the ambiguous sites of the consensus sequence were edited based on the alignment of the CloneIDs (see Figures 2-6) to generate the best possible sequence. The procedure was repeated for all six CloneID listed in Figure 1. This produced five unique contigs. The edited consensus sequences of the 5 contigs were imported into the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan). These consensus sequences were assembled. The contig 2511785 overlaps with contig 3506132, and this new contig was called 2535 (Figure 7). The contigs from the Sequencher program were copied into the Sequence Analysis software package of GCG.

Each contig was translated in all six reading frames into protein sequences. The *M. alpina* Δ5-desaturase (Ma29) and Δ6-desaturase (Ma524) sequences were compared with each of the translated contigs using the FastA search (a Pearson and Lipman

search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein)). Homology among these sequences suggest the open reading frames of each contig as underlined in Figures 3, 5, and 7. The homology among the *M. alpina* Δ5- and Δ6-desaturase sequences to contigs 2535 and 3854933 were utilized to create the final contig called 253538a (see Figure 8). Figure 9 is the FastA match of the translated sequences of the final contig 253538a and Ma29, and Figure 10 is the FastA match of the translated sequences of the final contig 253538a and Ma524.

Although the open reading frame was generated by merging the two contigs, the contig 2535 shows that there is a unique sequence in the beginning of this contig which does not match with the contig 3854933. Therefore, it is possible that these contigs were generated from independent desaturase-like human genes.

The contig 253538a contains an open reading frame encoding 432 amino acid (Figure 8, underlined). It starts with Gln (CAG) and ends with the stop codon (TGA) (both in bold). The contig 253538a aligns with both *M. alpina* Δ5- and Δ6-desaturase sequences, suggesting that it could be either of the desaturases, as well as other known desaturases which share homology with each other. The individual contigs listed in Figure 1, as well as the intermediate contig 2535 and the final contig 253538a can be utilized to isolate the complete genes for human desaturases.

Determination of Human Δ5-Desaturase Gene Sequence

Primers RO384 and RO388 were designed based on the 5′ and 3′ sequences, respectively, of contig 2535. The human monocyte cDNA library (Clontech, Palo Alto, CA) was amplified with the vector primer RO329 (5′- CAG ACC AAC TGG TAA TGG TAG - 3′) and

RO384 (5' - TCA GGC CCA AGC TGG ATG GCT GCA ACA TG - 3'), and also with the vector primer RO328 (5' - CTC CTG GAG CCC GTC AGT ATC - 3') and RO388 (5' - ATG GTG GGG AAG AGG TGG TGC TCA ATC TG - 3'). Polymerase Chain Reaction (PCR) was carried out in a 100 μl volume containing: 1 μl of human monocyte cDNA library, 10 pM each primer, 10 μl of 10X buffer and 1.0 U of Taq Polymerase. Thermocycler conditions in Perkin Elmer 9600 were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins. and 72 °C for 3 mins. PCR was followed by an additional extension at 72 °C for 7 minutes.

The PCR amplified mixture was run on a gel, and the amplified fragments were gel purified. The isolated fragment from PCR amplification with RO329 and RO384 was approximately 900 bp, and that from PCR amplification with RO328 and RO388 was approximately 650 bp. These isolated fragments were filled-in using T4 DNA polymerase, and the filled-in fragments were cloned into the PCR-Blunt vector (Invitrogen Corp., Carlsbad, CA). The clone of RO329/RO384 amplified fragment was designated as pRAE-7, and the clone of RO328/RO388 amplified fragment was designated as pRAE-8. Both ends of the clones were sequenced using ABI 373 DNA Sequencer (Applied Biosystems, Foster City, CA) and assembled using the Sequencher program (a sequence analysis program, Gene Codes Corporation, Ann Arbor, MI). This assembly of the sequences revealed that the two clones contained different sizes of the same gene (Figure 14). The complete sequence of the pRAE-7 gene was compiled (Figure 15) and searched against the known sequences in the public database.

The FastA algorithm is a Pearson and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein). The pRAE-7 gene sequence was translated in six reading frames, and using this method, the Swissprot database (Genetics Computer Group (GCG)

(Madison, WI) was searched. The gene in pRAE-7 was identified as a putative human desaturase based on its homology to known desaturases. The Swissprot database search produced matches against the omega-3 fatty acid desaturase from mung bean (23.4% identity in 303 AA overlap), linoleoyl-CoA desaturase from *Synechocystis* sp. (24.3% identity in 280 AA overlap), omega-6 fatty acid desaturase from soybean (19.7% identity in 284 AA overlap), and acyl-CoA desaturase 1 from *Saccharomyces cerevisiae* (21.6% identity in 134 AA overlap). The FastA search against the *M. alpina* desaturases produced matches against the Δ6-(31.9% identity in 285 AA overlap), the Δ5-(28.4% identity in 292 AA overlap), and the Δ12-(23.0% identity in 274 AA overlap) desaturases. The matched sequence alignment of the putative human desaturase gene in pRAE-7 against *M. alpina* Δ5-desaturase (Ma29), *M. alpina* Δ6-desaturase (Ma524) as well as to the contigs 2535 and 38 are displayed in Figures 16, 17, 18, and 19 respectively.

The contigs 2535, 38, and 253538a were generated based on assemblies of various sequences as well as their homologies against the known desaturases. However, upon examining Figures 18 and 19, it can be concluded that the contigs are merely indications as to what the sequences of the human desaturases might possibly be.

The 5' end of the gene, the ATG (Methionine), is necessary for expressing the human desaturase in yeast. Figures 16 and 17 show that pRAE-7 is probably just the last 2/3 of a desaturase gene. Several of the omega-3 and omega-6 fatty acid desaturases, as well as the linoleoyl-CoA desaturase mentioned above, are smaller than the *M. alpina* Δ5- and Δ6-desaturases, ranging in sizes of 359-380 amino acids. It was concluded from all of the sequences evaluated thus far that the isolated gene

probably needed anywhere from 180-480bp (60-160 amino acids) of additional 5' sequence for expressing a complete enzyme.

In order to extend the 5' sequence of the human desaturase gene, the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA) was used to screen the human liver marathon ready cDNA (Clontech). The rapid amplification of cDNA ends (RACE) reactions are efficient for both 5' and 3' long-distance PCR. Following the 5' RACE protocol outlined in the kit, the primers RO430 (5' - GTG GCT GTT GTT ATT GGT GAA GAT AGG CAT C - 3') (designed based on the pRAE-7 gene 3' sequence, downstream of the TAA (stop)) and the marathon adaptor primer(AP1) from the kit, were used to generate three PCR amplified products, which were designated A, B, and C. The fragment sizes were approximately 1.5 Kb, 1.4 Kb, 1.2 Kb, respectively. The fragments were filled-in with T4 DNA polymerase, and cloned into the pCR-blunt vector. A total of twenty-two clones were generated and sequenced. Using the FastA algorithm, the sequences were searched against the GenEMBL database of GCG.

Many of the sequences had a great homology to the human DNA sequence with the GenBank accession number of AC004228. This DNA sequence is described as: Sequencing in Progress, *Homo sapiens* Chromosome 11q12pac pDJ519o3; HTGS phase 1,18 unordered pieces. The 18 contigs were recorded in an arbitrary fashion. Using this sequence information and the information from the assembled sequences of the clones, the clones were categorized into five groups.

All of the clones have the same sequence downstream of the *BamHI* site (see Figure 12, underlined). But each group represents a different 5' sequence, with a total of 10 clones being too short to be the full length gene. Group 1, represented by clone A-1, is comprised of 5 clones which have homology to cytochrome b5 gene (Figure 20). A translational

start codon, ATG, is not present in clone A-1; however, as can be seen in Figure 21, there is an ATG (underlined) present in the ac004228 sequence 17 bp upstream of the strong area of homology between A-1 and ac004228. Starting from the strong area of homology, A-1 has an open reading frame of 1318 bp. However, starting from the ATG, the open reading frame is 1335 bp. Group 2, represented by clone 3-5, is comprised of 3 clones which have an ATG within an *NcoI* site, but four translational stop codons between the ATG and the *BamHI* site (Figure 22, the *NcoI*, *BamHI* sites are in bold, and the four termination codons are underlined). Group 3 is comprised of one clone, A-10, which has an ATG 135 bp upstream of the *BamHI* site, giving an open reading frame of 1267 bp (Figure 23). Group 4 is comprised of 2 clones, represented by clone A-16, which does not have an ATG; however, upstream of where the sequence aligns with ac004228, there is an ATG (Figure 24, underlined). The open reading frame of this group is 1347 bp. Group 5 is comprised of one clone which does not have an ATG. However, this clone matches the ac004228 sequence even upstream of the *BamHI* site (Figure 25).

As illustrated in Figure 26, many of the clones from the five groups are represented in order with the ac004228 sequence. There appeared to be a high level of splicing, with the sequence downstream of the *BamHI* site (in bold) acting as the common anchor for the various 5' exons. All of the potential start sites are also in bold, and the sequences found within the clones have been underlined.

The A-1 sequence was used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, CA, to see if its latest version would also have sequences with homology to our desaturase gene sequence. Two contigs were generated in this search, contig 3381584 and contig 2153526. The human desaturase gene sequence was initially compiled based on sequences from

Group I clones and ac004228. However, Figure 12 represents the actual DNA sequence of the isolated gene. The Incyte contigs were used to confirm this sequence (see Figures 27 and 28). The human desaturase translated sequence, consisting of 445 amino acids (Figure 13), was also matched with the original contigs 253538a and 38. These alignments are shown in Figures 29 and 30, respectively.

The FastA search of the human desaturase gene against the Swissprot database produced matches against the omega-3 fatty acid desaturase from mung bean (22.4% identity in 381 AA overlap), linoleoyl-CoA desaturase from *Synechocystis* Sp. (24.5% identity in 335 AA overlap), omega-6 fatty acid desaturase from soybean (20.3% identity in 290 AA overlap), and acyl-CoA desaturase 1 from *Saccharomyces cerevisiae* (21.4% identity in 168 AA overlap). The FastA search against *M. alpina* desaturases produced matches against the Δ6-(30.5% identity in 455 AA overlap), Δ5-(27.5% identity in 455 AA overlap), and Δ12-desaturases (22.5% identity in 382 AA overlap). The FastA match of the human desaturase translated sequence against the ma524 (*M. alpina* Δ6-desaturase) and ma29 (*M. alpina* Δ5-desaturase) sequences are shown in Figures 31 and 32, respectively.

EXAMPLE II

CONSTRUCTION OF CLONES

New clones were generated based on clones from three of the Groups mentioned above, clones A-1, A-10, and A-16. Two primers which were modified with 5' phosphate, RO526 (5'-CAT GGC CCC CGA CCC GGT GG-3') and RO527 (5'-GCG GCC ACC GGG TCG GGG GC-3'), were annealed together to form an adaptor. This adaptor which has *Nco*I and *Bsa*I overhangs, were ligated with the A-1 clone, which had been cut with *Bsa*I/*Hind*III and gel purified, for 15 min at room temperature. The pYX242(*Nco*I/*Hind*III) vector

(Novagen, Madison, WI) was added to this ligation mixture and allowed to incubate at room temperature for an additional 45 min. This produced a clone designated as pRAE-28-5. (Plasmid pRAE-28-5 was deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 on December 21, 1998, under the terms of the Budapest Treaty, and was accorded ATCC number _____.)

The A-10 clone was PCR amplified with RO512 (5'-GAT TGG GTG CCA TGG GGA TGC GGG ATG AAA AGG C-3') and RO5 (5'-GAA ACA GCT ATG ACC ATG-3'), the amplified product was cut with *Nco*I and *Hind*III and gel purified, and the purified fragment was cloned into pYX242 (*Nco*I/*Hind*III). This new clone was designated as pRAE-26-1.

The A-10 clone was also PCR amplified with RO580 (5' - TCC TGC GAA TTC ACC ATG AAA AGG CGG GAG AGA G - 3') and RO5, the amplified product was cut with *Nco*I and *Hind*III and gel purified, and the purified fragment was cloned into pYX242 (*Nco*I/*Hind*III). This new clone was designated as pRAE-33.

Two primers which were modified with 5' phosphate, RO578 (5' - CAT GGC TAG GAG AGG CAG CGC AGC CGC GTC TGG AC - 3') and RO579 (5' - CTA GGT CCA GAC GCG GCT GCG CTG CCT CTC CTA GC - 3'), were annealed together to form an adaptor. This adaptor which has *Nco*I and *Bln*I overhangs, were ligated with the A-16 clone, which had been cut with *Bln*I/*Hind*III and gel purified, for 15 min at room temperature. The pYX242(*Nco*I/*Hind*III) vector was added to this ligation mixture and allowed to incubate at room temperature for an additional 45 min. This produced a clone designated as pRAE-35.

EXAMPLE III

EXPRESSION OF HUMAN Δ5-DESATURASE

The constructs pRAE-26-1, pRAE-28-5, pRAE-33, and pRAE-35 were transformed into *S. cerevisiae* 334 and screened for desaturase activity. The substrates DGLA (20:3n-6), OA(18:1n-9), AA(20:4n-6), and LA(18:2n-6) were used to determine the activity of the expressed gene from constructs pRAE-26-1 and pRAE-28-5. Only the substrate DGLA was used to determine the activity of the expressed gene from all of the constructs. The negative control strain was *S. cerevisiae* 334 containing the unaltered pYX242 vector. The cultures were grown for 48 hours at 30°C, in selective media (Ausubel *et al.*, Short Protocols in Molecular Biology, Ch. 13, P. 3-5 (1992)), in the presence of a particular substrate. Lipid fractions of each culture were extracted for analysis. The desaturase activity results are provided in Figures 33 and 34.

All of the values in Figure 33 are the average of two separate samples per strain, tested in the same run. The substrate, as well as the fatty acid it was converted to, is shown in bold. The expressed gene in the strain 334 (pRAE-28-5) is a Δ5-desaturase. It converted the substrate DGLA to a higher percent of AA than the control strain 334(pYX242), 0.127% vs. 0.062%, respectively. The percent of AA present in the cultures of strains 334(pRAE-26-1), 334(pRAE-33), and 334(pRAE-35) are comparable to that of the control strain (0.075%, 0.062%, and 0.063%, respectively). Therefore, it can be concluded that the cyt b5 sequence containing gene in the construct pRAE-28-5 expresses an active human Δ5-desaturase; whereas, the other variations of the gene do not.

The activity of the human Δ5-desaturase was further confirmed in the experiment outlined in Figure 34. Included in

this figure are the fatty acid profiles of the strains 334(pRAE-28-5), 334(pRAE-26-1), and the control strain 334(pYX242) when DGLA(20:3n-6), OA(18:1n-9), AA(20:4n-6), or LA(18:2n-6) was used as the substrate, as well as when no substrate was added. Again, the strain 334(pRAE-28-5) expressed an active human Δ5-desaturase, converting DGLA to AA at a higher percent than the control strain, 0.106% vs. 0.065%, respectively. The strain 334(pRAE-26-1) had about the same amount of AA (0.06%) as the control. The conversion of the substrate OA to LA was not detected, confirming that the strains do not have a Δ12-desaturase activity. The conversion of the substrate AA to eicosapentaenoic acid (EPA, 20:5n-3) was detected, but at a very low level equal to that of the control strain, confirming that the strains do not have a Δ17-desaturase activity. The conversion of the substrate LA to GLA was detected, but again at a very low level equal to the control strain, confirming that the strains do not have a Δ6-desaturase activity.

The present sequence (Figure 12) differs from the Genbank sequence g3169158 of the LifeSeq database with respect to two positions. In particular, with respect to the nucleotide sequence of sequence g3169158, position 1082 is an adenosine; however, in the present sequence position 1082 is a thymine (see Figure 12). Furthermore, position 1229 of sequence g3169158 is an adenine whereas in the present sequence position 1229 is a guanine. In terms of an amino acid sequence comparison, position 361 of the present sequence is a leucine (see Figure 13), and position 361 of sequence g3169158 is a glutamine. Furthermore, position 410 of the present sequence is an arginine, whereas position 410 of sequence g3169158 is a histidine. Additionally, sequence g3169158 is described, in the database, as a "hypothetical protein" which "exhibits similarity to motifs found in delta 6 desaturase, a hypothetical cytochrome

b5 containing fusion protein." However, as demonstrated in the above example, the protein encoded by the sequence in Figure 12 is a human Δ5-desaturase, not a Δ6-desaturase.

EXAMPLE IV

EXPRESSION OF HUMAN Δ5-DESATURASE IN INSECT CELLS

Insect cells were used as another eukaryotic host for expression of the human Δ5-desaturase. The baculovirus expression system involves the use of insect cells to express a gene, in this case, the human Δ5-desaturase, which has been cloned into a baculovirus expression vector. Insect cells are known to have no endogenous PUFA desaturase activities. Therefore, this system is suitable for expression and characterization of the recombinant desaturases.

The fragment containing the human Δ5-desaturase gene (pRAE-28-5, see EXAMPLE II) was PCR amplified using Expand High Fidelity PCR System (Boehringer Mannheim Corp., Indianapolis, IN) and a set of primers containing appropriate restriction sites. The upstream primer designated RO676 (5'- ATA CGT GAA TTC GCC GCC ACC ATG GCC CCC GAC CCG GTG - 3') corresponded to the sense strand of Δ5 cDNA and contained an EcoRI site 5' upstream of the ATG. The downstream primer RO677 (5' - TAT CCG CTC GAG TTA TTG GTG AAG ATA GGC ATC TAG - 3') corresponded to the antisense strand at the 3' end of the Δ5 cDNA, and included an XhoI site immediately downstream of the translational termination codon. The PRC reaction, in a final volume of 100 μl, was carried out as follows: 5 mins denaturation at 94 °C, then 45 seconds at 94 °C, 45 seconds at 55 °C and 2 min at 72 °C for 30 cycles, and 7 mins. extension at 72 °C at the end of the amplification. The human Δ5 PCR amplified product was analyzed by agarose-gel electrophoresis, gel purified, digested with

EcoRI and XhoI, and then ligated into pFastBac1 baculovirus donor plasmid (Gibco-BRL, Gaithersburg, MD) which was restricted with the same enzymes. The respective baculovirus clone was designated as pJPBh4 for the human Δ5-desaturase. This pFastBac1 vector contains an expression cassette which has a polyhedrin promoter, a SV40 polyadenylation signal, and a gentamycin resistance marker.

The initial transformation was done in XL1 blue cells (Invitrogen, Carlsbad, CA). Positive clones were then transformed into E. coli DH10Bac (Gibco-BRL, Gaithersburg, MD) which contains the baculovirus genome. The positive clones were selected by blue white screening in which white colonies contain the recombinant bacmid. White colonies were then selected for bacmid DNA isolation. DNA was isolated using a Qiagen plasmid isolation kit (Qiagen, Inc., Valencia, CA), specific for DNA over 135 kb long. The recombinant bacmid DNA was analyzed on a 0.6% agarose gel to confirm the presence of the high molecular weight DNA. PCR analysis, using pUC/M13 primers (forward 5' - TGT AAA ACG ACG GCC AGT - 3' and reverse 5'- GAA ACA GCT ATG ACC ATG - 3') was also performed to confirm the correct insert size for the desaturase cDNA within the bacmid.

The Sf9 insect cells (Spodoptera frugiperda) were used for the recombinant bacmid DNA transfection. These cells were grown in serum free media (Gibco-BRL, Gaithersburg, MD). Transfection was carried out according to the CellFECTIN Sf900 protocol (Gibco-BRL, Gaithersburg, MD). The recombinant virus was recovered by collecting the supernatant at 72 hours post-transfection. A plaque assay was performed on the supernatant to determine the titer of recovered recombinant virion particles. A recombinant viral stock was made for the expression studies. All infections with the recombinant virus were done during the mid-logarithmic growth phase of the Sf9's

and infected at 5 MOI (Multiplicity of Infection). To analyze the activity of the expressed human Δ5-desaturase gene, the Sf9m cells were plated at a concentration of 1 x 10^6 cells/well in a 6-well tissue culture plate and infected with 100 μl of the virus stock (approximately 5 MOI). The substrate, dihomo-gamma-linolenic acid (DGLA, C20:3n-6) was supplemented at the time of infection, at a concentration of 100 μM. A mock infected Sf9, as well as cells infected with a recombinant virus containing the GusA reporter gene, were used as negative controls in each experiment. The medium was collected 48 hours post infection and saved. The cells were collected and submitted for lipid analysis.

For fatty acid analysis, cell pellets were vortexed with 6 ml of methanol, followed by the addition of 12 ml of chloroform and tridecanoin (as internal standard). The mixtures were incubated for at least one hour at room temperature or at 4 °C overnight. The chloroform layer was extracted and filtered through a Whatman filter with one gram of anhydrous sodium sulfate to remove particulates and residual water. The organic solvents were evaporated at 40 °C under a stream of nitrogen. The extracted lipids were derivatized to fatty acid methyl esters (FAME) for gas chromatography analysis (GC) by adding 2 ml of 0.5 N potassium hydroxide in methanol to a closed tube. The samples were heated at 95 to 100 °C for 30 minutes and cooled to room temperature. Approximately 2 ml of the 14% boron trifluoride in methanol was added and the heating repeated. After the extracted lipid mixture cooled, 2 ml of water and 1 ml of hexane were added to extract the FAME for GC analysis. The percent conversion was calculated by dividing the product produced by the sum of (the product produced and the substrate) and then multiplying by 100.

The fatty acid synthesis in insect cells infected with recombinant virus containing the human Δ5 cDNA is summarized in Table 1. The conversion of the added substrate, DGLA (C20:3n-6), to arachidonic acid (AA, 20:4n-6) was monitored. The quantity of arachidonic acid (AA, 20:4n-6) produced by the human Δ5-desaturase was 9.67% of the total fatty acid versus the control which did not produce any AA. This resulted in a 29.6% conversion of DGLA to AA.

These data indicate that the human Δ5-desaturase can be expressed in another eukaryotic host (insect cells) in a biologically active form as demonstrated by the production of AA.

Table 1

Fatty Acid		Human Δ5	Control
18:1n-9		19.15	19.99
18:3n-6		2.43	5.18
*20:3n-6		22.95	30.00
20:4n-6	(29.6%)	9.67	ND
22:1n-9		0.11	0.25

* indicates substrate added

ND indicates None Detected

Nutritional Compositions

The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:
-Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
-Lactose-free formulation to avoid lactose-associated diarrhea.
-Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.
-Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
-1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
-Recommended levels of vitamins and minerals.
-Vegetable oils to provide recommended levels of essential fatty acids.
-Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and disglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric

sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

Features:
-First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.
-Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.
-Nutritionally complete to meet the nutritional needs of the infant.
-Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.
-Lactose-free formulation to avoid lactose-associated diarrhea.
-Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.
-Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
-Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.
-1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11% calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

C. Isomil® SF Sucrose-Free Soy Formula With Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:
-Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
-Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
-Sucrose free for the patient who cannot tolerate sucrose.
-Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
-1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
-Recommended levels of vitamins and minerals.
-Vegetable oils to provide recommended levels of essential fatty acids.
-Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 75% water, 11.8% hydrolized cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0. 17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, abscorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride,

carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate,L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

D. Isomil® 20 Soy Formula With Iron Ready To Feed, 20 Cal/fl oz.:

Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0. 11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, abscorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E. Similac® Infant Formula:

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a

supplement to breastfeeding is needed or as a routine feeding if breastfeeding is not adopted.

Features:
-Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.
-Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.
-Carbohydrate as lactose in proportion similar to that of human milk.
-Low renal solute load to minimize stress on developing organs.
-Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, abscorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete formula developed to

provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:
-Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
-Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
-Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.
-More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

G. Similac Natural Care Low-Iron Human Milk Fortifier Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

II. NUTRITIONAL FORMULATIONS

A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-

cholesterol diets. Although it is primarily an oral supplement, it can be fed by tube.

Patient Conditions:
-For patients on modified diets
-For elderly patients at nutrition risk
-For patients with involuntary weight loss
-For patients recovering from illness or surgery
-For patients who need a low-residue diet

Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS:

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-rich alternative to other snacks. ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

Patient Conditions:
-For patients who need extra calories, protein, vitamins and minerals.
-Especially useful for people who do not take in enough calories and nutrients.
-For people who have the ability to chew and swallow
-Not to be used by anyone with a peanut allergy or any type of allergy to nuts.
Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.

Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

Soy protein isolate	74%
Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

Partially hydrogenated cottonseed and soybean oil	76%
Canola oil	8%
High-oleic safflower oil	8%
Corn oil	4%
Soy lecithin	4%

Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

High-fructose corn syrup	24%
Brown sugar	21%
Maltodextrin	12%
Honey	11%
Crisp rice	9%
Glycerine	9%
Soy Polysaccharide	7%
Oat bran	7%

C. ENSURE® HIGH PROTEIN:

Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:
-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features:
-Low in saturated fat
-Contains 6 g of total fat and < 5 mg of cholesterol per serving
-Rich, creamy taste
-Excellent source of protein, calcium, and other essential vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-OIeic Safflower

Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Suffate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	85%
Soy protein isolate	15%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and soy.

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories,

with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	60%
Maltodextrin	40%

Chocolate:

Sucrose	70%
Maltodextrin	30%

D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.
-For healthy adults who don't eat right and need extra nutrition.
Features:

-Low in fat and saturated fat
-Contains 3 g of total fat per serving and < 5 mg cholesterol
-Rich, creamy taste
-Excellent source of calcium and other essential vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients:

French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine

Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:
The protein source is calcium caseinate.

Calcium caseinate	100%

Fat:

The fat source is a blend of two oils: high-oleic safflower and canola.

High-oleic safflower oil	70%
Canola oil	30%

The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the, calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:
ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and

orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	51%
Maltodextrin	49%

Chocolate:

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

Patient Conditions:

-For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
-For patients who need to gain or maintain healthy weight

Features:
-Rich, creamy taste
-Good source of essential vitamins and minerals

Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%

Carbohydrate:

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, buffer pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz. Coffee flavor contains a trace amount of caffeine.

F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

Patient Conditions:
-For patients with increased calorie and protein needs, such as following surgery or injury.
-For patients with limited volume tolerance and early satiety.

Features:
-For supplemental or total nutrition
-For oral or tube feeding
-1.5 CaVmL,
-High nitrogen
-Calorically dense

Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,

Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:
-For patients on modified diets
-For elderly patients at nutrition risk
-For patients recovering from illness/surgery
-For patients who need a low-residue diet

Features:
-Convenient, easy to mix
-Low in saturated fat
-Contains 9 g of total fat and < 5 mg of cholesterol per serving
-High in vitamins and minerals
-For low-cholesterol diets
-Lactose-free, easily digested

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy

Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%

Carbohydrate:

ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla:

Corn Syrup	35%
Maltodextrin	35%
Sucrose	30%

H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:
-For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
-For patients with swallowing impairments

Features:
-Rich and creamy, good taste
-Good source of essential vitamins and minerals
-Convenient-needs no refrigeration
-Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:
Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate,

Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is nonfat milk.

Nonfat milk	100%

Fat:

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil	100%

Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

Sucrose	56%
Lactose	27%
Modified food starch	17%

Chocolate:

Sucrose	58%
Lactose	26%
Modified food starch	16%

I. ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For patients who can benefit from increased dietary fiber and nutrients

Features:

-New advanced formula-low in saturated fat, higher in vitamins and minerals
-Contains 6 g of total fat and < 5 mg of cholesterol per serving
-Rich, creamy taste
-Good source of fiber
-Excellent source of essential vitamins and minerals
-For low-cholesterol diets
-Lactose- and gluten-free

Ingredients:

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride, Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates	80%
Soy protein isolate	20%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

High-oleic safflower oil	40%
Canola oil	40%

Corn oil	20%

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq$ 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and $\leq$ 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
Oat Fiber	7%
Soy Fiber	2%

Chocolate:

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

Fiber:

The fiber blend used in ENSURE WITH FIBER consists of oat fiber and soy polysaccharide. This blend results in approximately 4 grams of total dietary fiber per 8-fl. oz can. The ratio of insoluble to soluble fiber is 95:5.

The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

J. Oxepa™ Nutritional Product

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil), γ-linolenic acid (GLA from borage oil), and elevated antioxidant levels.

Caloric Distribution:

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs. The distribution of Calories in Oxepa is shown in Table IV.

Table IV. Caloric Distribution of Oxepa

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1
Protein (g)	14.8	62.5	16.7

Water (g)	186	785	---

Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

-Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain trigy1cerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

Table V. Typical Fatty Acid Profile

	% Total Fatty Acids	g/8 fl oz*	9/L*
Caproic (6:0)	0.2	0.04	0.18
Caprylic (8:0)	14.69	3.1	13.07
Capric (10:0)	11.06	2.33	9.87
Palmitic (16:0)	5.59	1.18	4.98
Palmitoleic	1.82	0.38	1.62
Stearic	1.94	0.39	1.64
Oleic	24.44	5.16	21.75
Linoleic	16.28	3.44	14.49
α-Linolenic	3.47	0.73	3.09
γ-Linolenic	4.82	1.02	4.29
Eicosapentaenoic	5.11	1.08	4.55
n-3-Docosapent-aenoic	0.55	0.12	0.49
Docosahexaenoic	2.27	0.48	2.02
Others	7.55	1.52	6.72

Fatty acids equal approximately 95% of total fat.

Table VI. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz
	40.1 mg/L

Carbohydrate:

-The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).

-The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.

-The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (C02) production. High C02 levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.

-Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa

is also sufficient to minimize gluconeogenesis, if energy needs are being met.

Protein:

-Oxepa contains 14.8 g of protein per 8-fl-oz serving (62.5 g/L).

-The total calorie/nitrogen ratio (150:1) meets the need of stressed patients.

-Oxepa provides enough protein to promote anabolism and the maintenance of lean body mass without precipitating respiratory problems. High protein intakes are a concern in patients with respiratory insufficiency. Although protein has little effect on C02 production, a high protein diet will increase ventilatory drive.

-The protein sources of Oxepa are 86.8% sodium caseinate and 13.2% calcium caseinate.

- The amino acid profile of the protein system in Oxepa meets or surpasses the standard for high quality protein set by the National Academy of Sciences.

* Oxepa is gluten-free.

CLAIMS:

1. An isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence represented by SEQ ID NO:1 (Figure 12).

2. The isolated nucleotide sequence of claim 1 wherein said sequence is represented by SEQ ID NO:1.

3. The isolated nucleotide sequence of claims 1 or 2 wherein said sequence encodes a functionally active desaturase which utilizes a polyunsaturated fatty acid as a substrate.

4. The nucleotide sequence of claim 1 wherein said sequence is derived from a mammal.

5. The nucleotide sequence of claim 4 wherein said Sequence is derived from a human.

6. A purified protein encoded by said nucleotide sequence of claims 1 or 2.

7. A purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of said purified protein of claim 6.

8. A method of producing a human Δ5-desaturase comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 12);

b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;

c) introducing said vector into a host cell under time and conditions sufficient for expression of said human Δ5-desaturase.

9. The method of claim 8 wherein said host cell is a eukaryotic cell or a prokaryotic cell.

10. The method of claim 9 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

11. The method of claim 9 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

12. The method of claim 11 wherein said fungal cell is a yeast cell.

13. The method of claim 12 wherein said yeast cell is selected from the group consisting of Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. and Pichia spp.

14. The method of claim 13 wherein said yeast cell is Saccharomyces cerevisiae.

15. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 12) operably linked to b) a promoter.

16. A host cell comprising said vector of claim 15.

17. The host cell of claim 16, wherein said host cell is a eukaryotic cell or a prokaryotic cell.

18. The host cell of claim 17 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

19. The host cell of claim 17 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

20. The host cell of claim 19 wherein said fungal cell is a yeast cell.

21. The host cell of claim 20 wherein said yeast cell is selected from the group consisting of Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. and Pichia spp.

22. The host cell of claim 21 wherein said host cell is Saccharomyces cerevisiae.

23. A plant cell, plant or plant tissue comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid by said plant cell or tissue.

24. The plant cell, plant or plant tissue of claim 23 wherein said polyunsaturated fatty acid is AA or EPA.

25. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 23.

26. A transgenic plant comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

27. A mammalian cell comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of altered levels of AA or EPA when said cell is grown in a culture media comprising a fatty acid selected from the group consisting of an essential fatty acid, LA and ALA.

28. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding a human Δ5-desaturase operably linked to a promoter.

29. The transgenic, non-human mammal of claim 28, wherein said DNA sequence is represented by SEQ ID NO:1 (Figure 12).

30. A fluid produced by said transgenic, non-human mammal of claim 29 wherein said fluid comprises a detectable level of at least human Δ5-desaturase.

31. A method for producing a polyunsaturated fatty acid comprising the steps of:

a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 12);
b) constructing a vector comprising said isolated nucleotide sequence;
c) introducing said vector into a host cell under time and conditions sufficient for expression of said human Δ5-desaturase enzyme; and
d) exposing said expressed human Δ5-desaturase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

32. The method according to claim 31, wherein said substrate polyunsaturated fatty acid is DGLA or 20:4n-3 and said product polyunsaturated fatty acid is AA or EPA, respectively.

33. The method according to claim 31 further comprising the step of exposing said product polyunsaturated fatty acid to an elongase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

34. The method according to claim 33 wherein said product polyunsaturated fatty acid is AA or EPA and said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid, respectively.

35. The method of claim 33 further comprising the steps of exposing said another polyunsaturated fatty acid to an additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

36. The method of claim 35 wherein said final polyunsaturated fatty acid is (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

37. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33, and said final polyunsaturated fatty acid produced according to the method of claim 35.

38. The nutritional composition of claim 37 wherein said product polyunsaturated fatty acid is AA or EPA.

39. The nutritional composition of claim 37 wherein said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid.

40. The nutritional composition of claim 37 wherein said final polyunsaturated fatty acid is DHA.

41. The nutritional composition of claim 37 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

42. The nutritional composition of claim 41 wherein said nutritional composition is administered to a human or an animal.

43. The nutritional composition of claim 42 wherein said nutritional composition is administered enterally or parenterally.

44. The nutritional composition of claim 41 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and protein hydrolysates.

45. The nutritional composition of claim 44 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

46. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33, and said final polyunsaturated fatty acid produced according to the method of claim 35 and 2) a pharmaceutically acceptable carrier.

47. The pharmaceutical composition of claim 46 wherein said pharmaceutical composition is administered to a human or an animal.

48. The pharmaceutical composition of claim 46 wherein said pharmaceutical composition further comprises an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

49. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33 and said final polyunsaturated fatty acid produced according to the method of claim 35.

50. The animal feed of claim 49 wherein said

product polyunsaturated fatty acid is AA or EPA.

51. The animal feed of claim 49 wherein said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid.

52. The animal feed of claim 49 wherein said final polyunsaturated fatty acid is (n-6)-docosapentaenoic acid or DHA.

53. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33 and said final polyunsaturated fatty acid produced according to the method of claim 35.

54. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 37 in an amount sufficient to effect said treatment.

55. The method of claim 11 wherein said insect cell is Spodoptera frugiperda.

56. The method of claim 19 wherein said insect cell is Spodoptera frugiperda.

Sections of the Desaturases	Clone ID from Incyte LifeSeq Database	Keyword
151-300 delta 5	3808675	fatty acid desaturase
301-446 delta 5	354535	delta 6
151-300 delta 6	3448789	delta 6
151-300 delta 6	1362863	delta 6
151-300 delta 6	2394760	delta 6
301-457 delta 6	3350263	delta 6

Figure 1

Edited Contig 2692004

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Figure 2

Edited Contig 2153526

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Figure 3

Edited Contig 3506132

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Figure 4

Edited Contig 3854933

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Figure 5

Edited Contig 2511785

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Figure 6

Contig 2535

Figure 7

Edited Contig 253538a

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Figure 8

FastA Match of Ma29 and contig 253538a

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Smith-Waterman score: 408;    27.0% identity in 441 aa overlap

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Ma29.pep      MGTDQGKT---FTWEELAAHNTKDDLLLAIRGRVYDVTKFLSRHPGGVDTLLLGAGRDVT
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253538a          QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVISHYAGQDAT
                          10        20        30        40        50

              60        70        80        90       100       110
Ma29.pep      PVFEMYHAF-GAADAIMKKYYVGTLVSNELPIFPEPTVFHKTIKTRVEGYFTDRNIDPKN
                |  :|   | :   |::  :| | | | | | |||  ::       |   | : :  :
253538a       DPFVAFHINKGLVKKYMNSLLIGEL-SPEQPSF-EPTKNKELTDEFRELRATVERMGLMK
                60        70        80          90       100       110

             120       130       140       150       160       170
Ma29.pep      RPEIWGRYALIFGSLIASYYAQLFVPFVVERTWLQVVF-AIIMGFACAQVGLNPLHDASH
                :::  : | :  ::    |  :: :|   ::|  :: |:::: : ||:|    || :|
253538a       ANHVF--FLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQ-HDYGH
                   120       130       140       150       160       170

              180       190           200       210       220
Ma29.pep      FSVTHNPTVWKILGATHDF----FNGASYLVWMYQHMLGHHPYTNIAGADPDVSTSE---
              :|| ::|  |: |  :| |    ::|||   | ::| : ||   ||   ||||:  :
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                     180       190       200       210       220

              230       240       250       260       270       280
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                   ::  | : |::  || ::::|:  | |   : |: :|    |: ::  :: : :
253538a       LGEWQPIEYGKKKLKYLPYNHQHEYFFLIGPPLLIPMYFQYQI----IMTMIVHKNWVDL
              230       240       250       260       270       280

                   290       300       310       320       330       340
Ma29.pep      NPISTWHTVMFWGGKAFFVWYRLIVPLQYLPLGKVLLLFTVADMVSSYWLALTFQANHVV
                 :| : ::     ||: |   :|: |  || :||:::   :: |:|:: : | ||:|
253538a       ----AWAVSYYI---RFFITY---IPF-YGILG-ALLFLNFIRFLESHWFVWVTQMNHIV
                       290       300       310       320       330

                   350       360       370       380       390
Ma29.pep      EEVQWPLPDENGIIQKDWAAMQVETT----QDYAHDSHLWTSITGSLNYQAVHHLFPNVS
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253538a       MEI-----DQEAY--RDWFSSQLTATCNVEQSFFND---WFS--GHLNFQIEHHLFPTMP
                          340       350       360           370

              400       410       420       430       440
Ma29.pep      QHHYPDILAIIKNTCSEYKVPYLVKDTFWQAFASHLEHLRVLGLRPKEEX
              :|:   |  ::|: |::: : |  |
253538a       RHNLHKIAPLVKSLCAKHGIEYQEKPLLRALLDIIRSLKKSGKLWLDAYLHKX
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Figure 9

FastA Match of Ma524 and contig 253538a

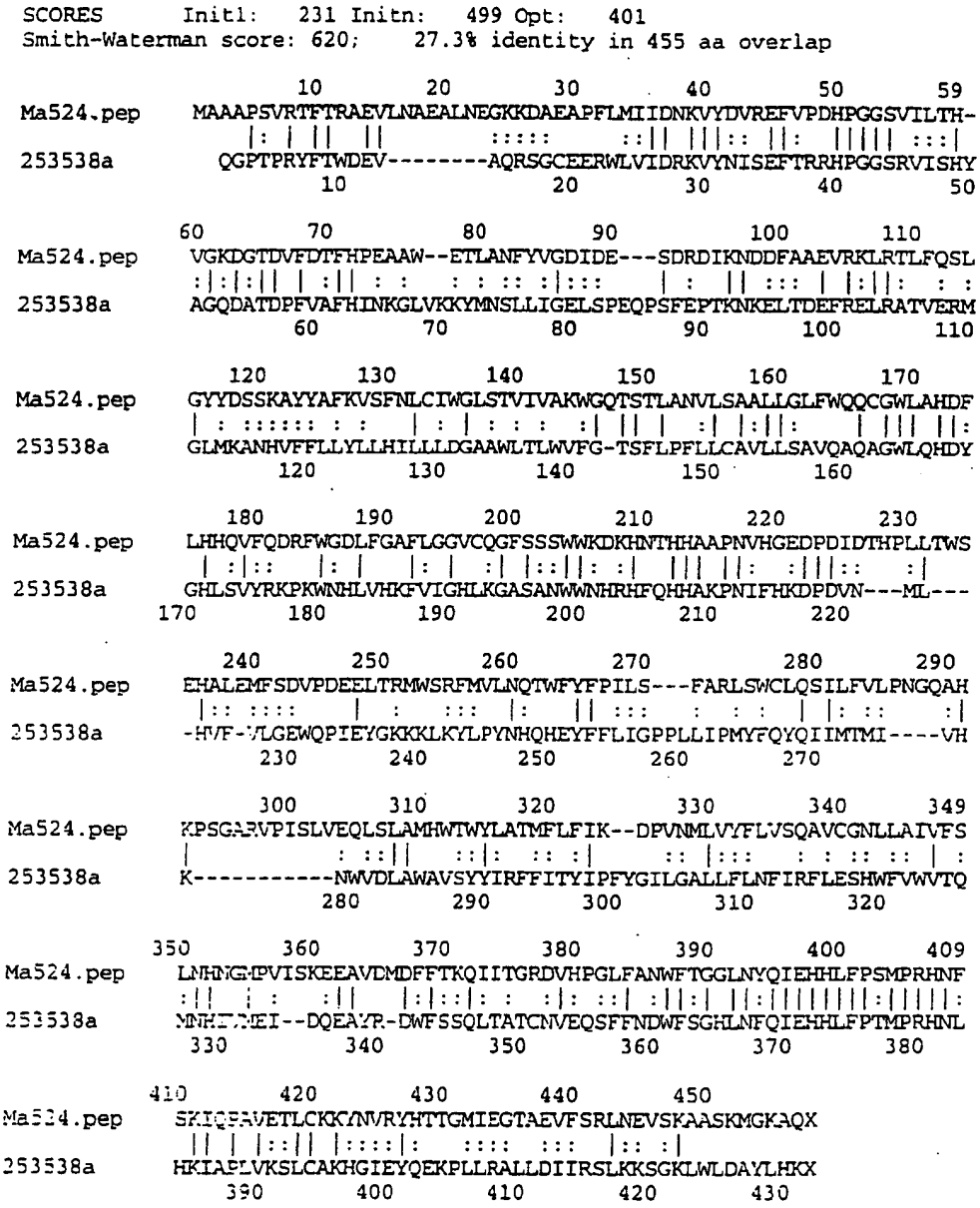
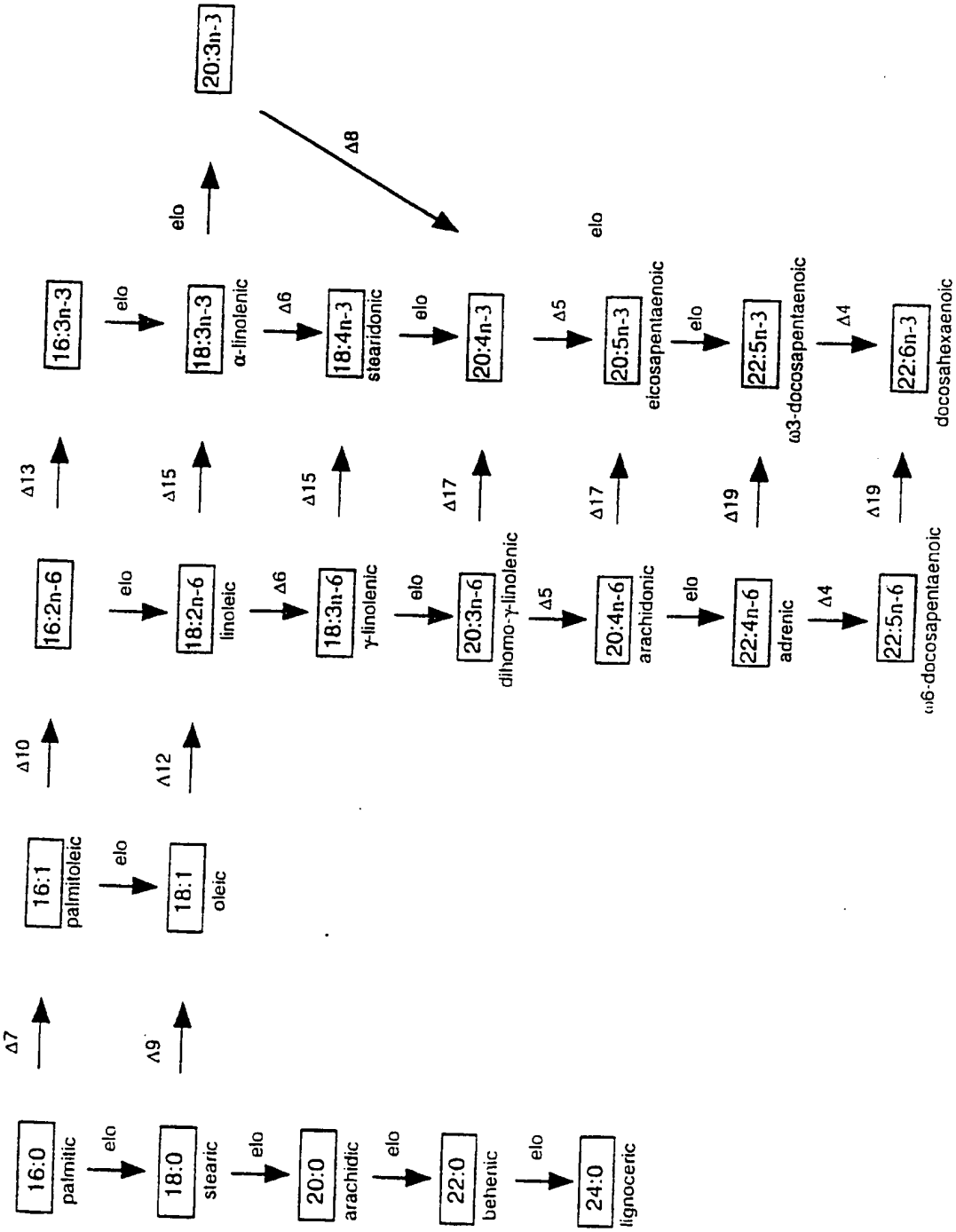


Figure 10

Figure 11

Fatty Acid Biosynthesis Pathways



Human Δ5-desaturase

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Figure 12

Human Δ5-desaturase

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151  WVFGTSFLPF LLCAVLLSAV QAQAGWLQHD FGHLSVFSTS KWNHLLHHFV
201  IGHLKGAPAS WWNHMHFQHH AKPNCFRKDP DINMHPFFFA LGKILSVELG
251  KQKKKYMPYN HQHKYFFLIG PPALLPLYFQ WYIFYFVIQR KKWVDLAWMI
301  TFYVRFFLTY VPLLGLKAFL GLFFIVRFLE SNWFVWVTQM NHIPMHIDHD
351  RNMDWVSTQL LATCNVHKSA FNDWFSGHLN FQIEHHLFPT MPRHNYHKVA
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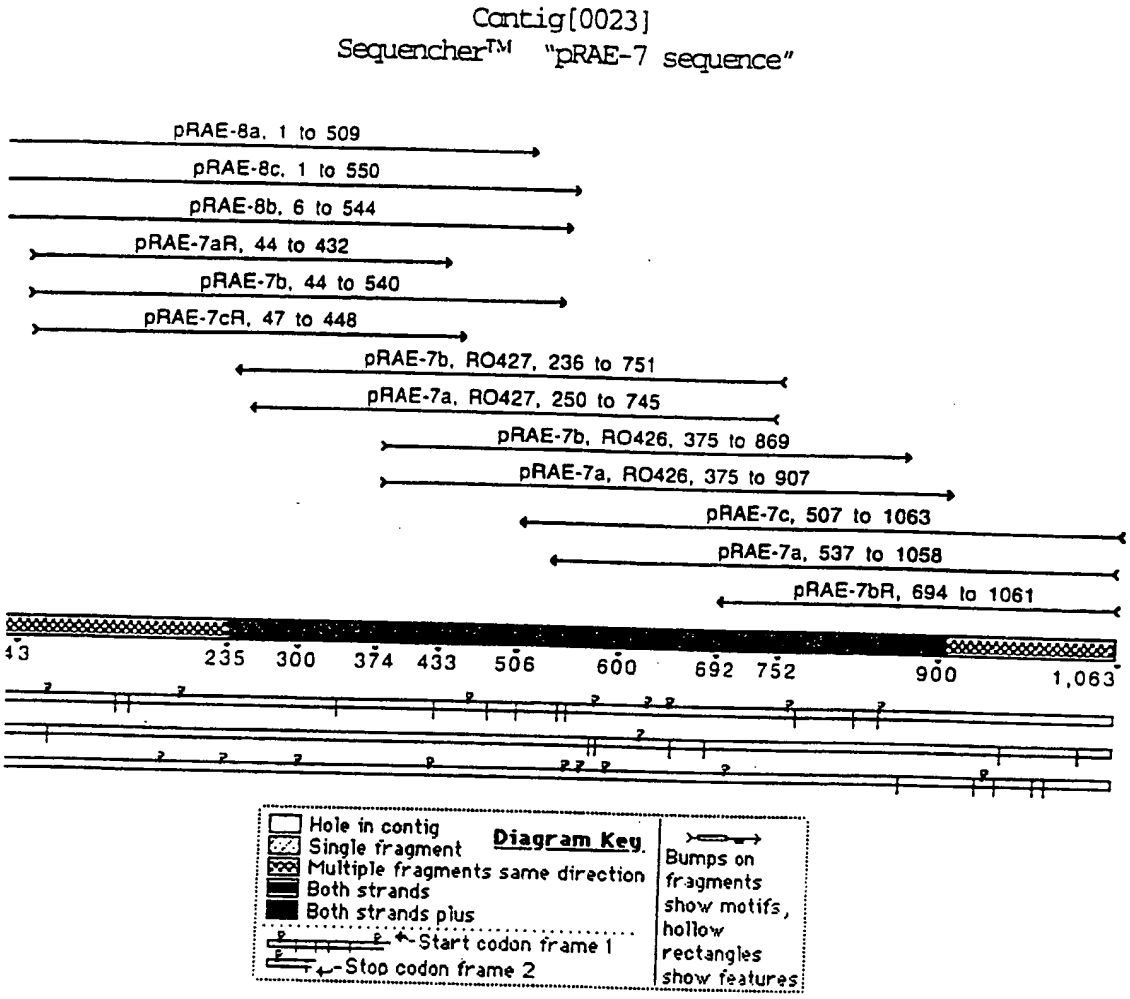
Figure 13


Contig[0023]
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pRAE-7b, 44 to 540
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pRAE-7b, RO426, 375 to 869
pRAE-7a, RO426, 375 to 907
pRAE-7c, 507 to 1063
pRAE-7a, 537 to 1058
pRAE-7bR, 694 to 1061
43
235
300
374
433
506
600
692
752
900
1,063
Diagram Key
Hole in contig
Single fragment
Multiple fragments same direction
Both strands
Both strands plus
Start codon frame 1
Stop codon frame 2
Bumps on fragments show motifs, hollow rectangles show features

Figure 14

pRAE-7 Complete Sequence

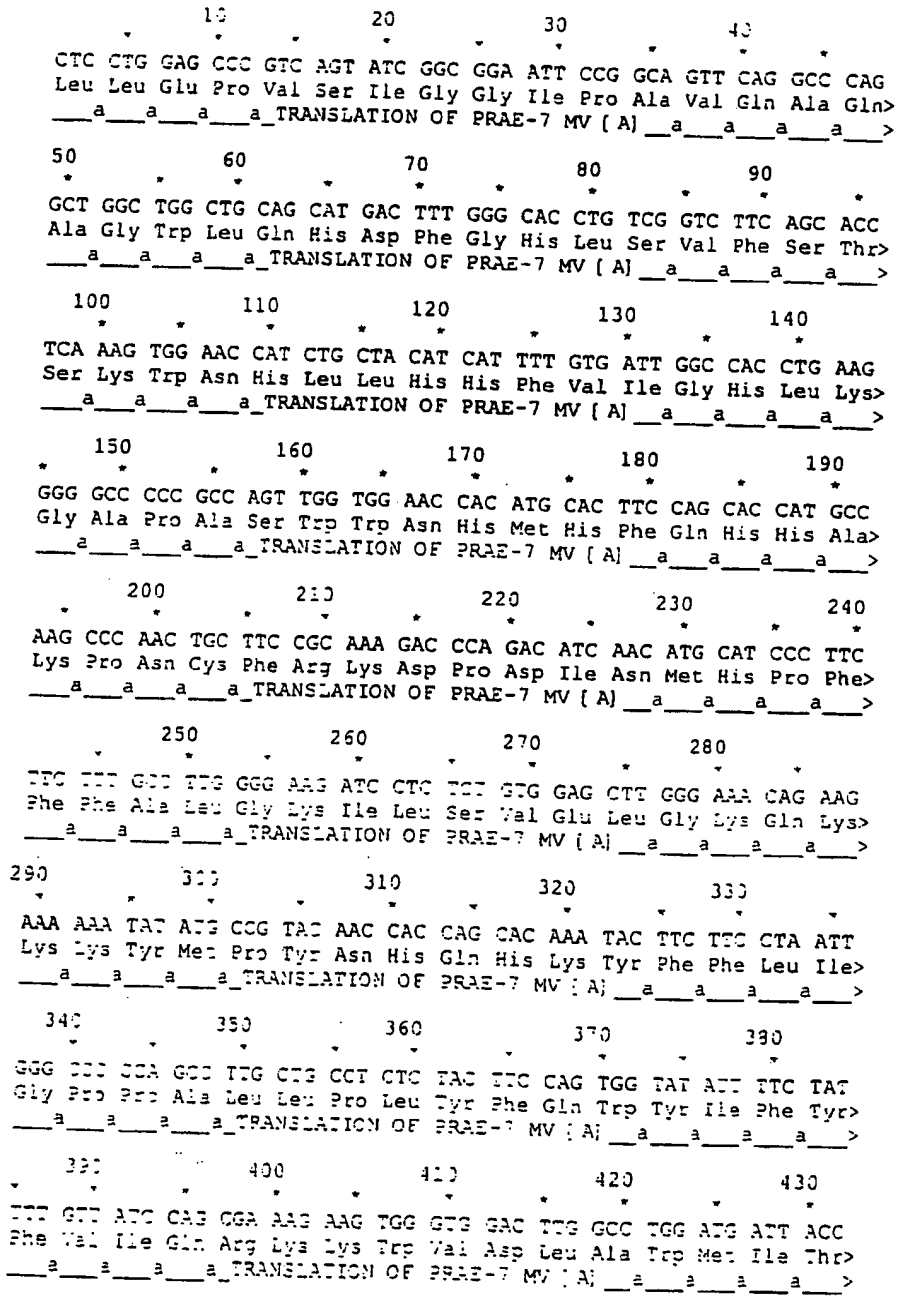
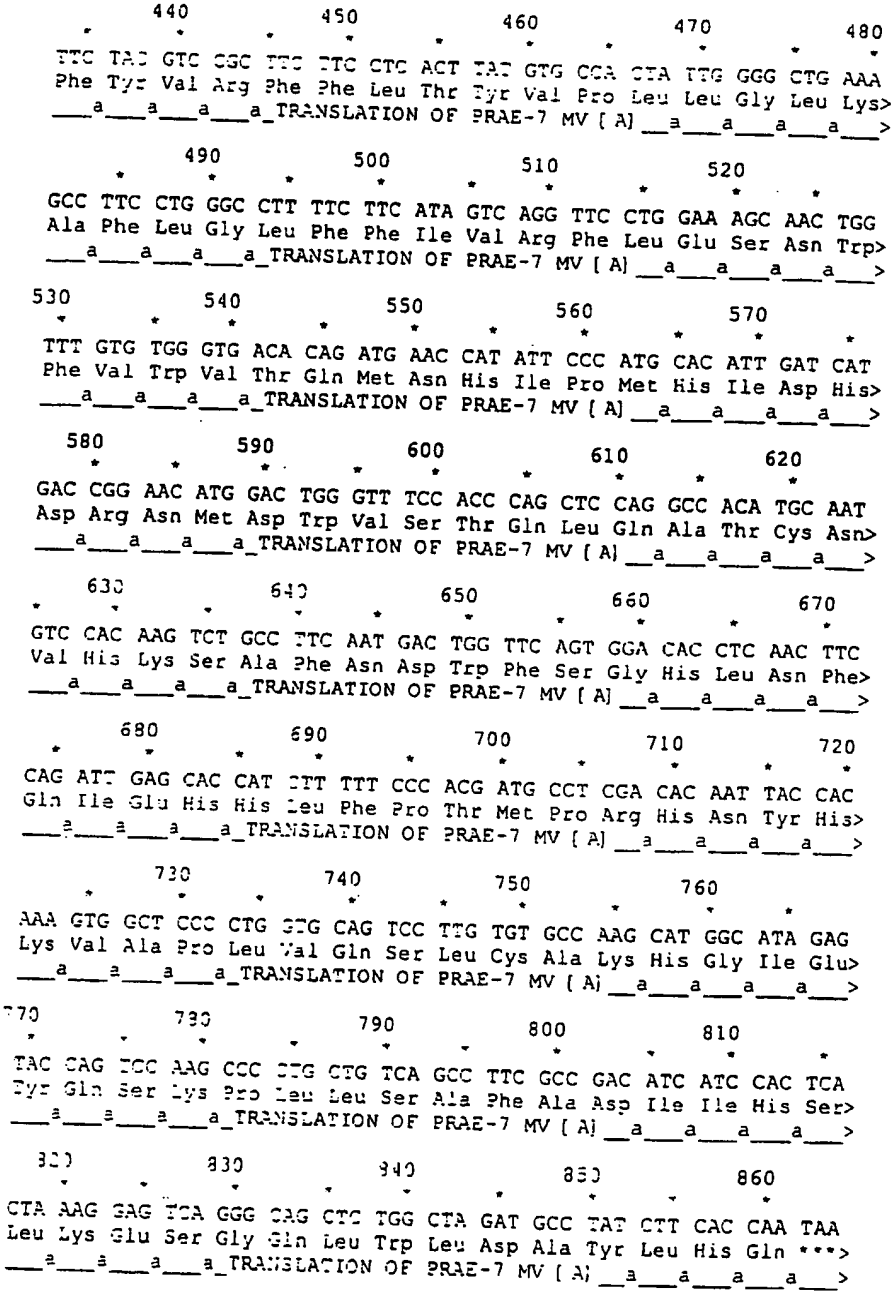


Figure 15a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Ala Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp>
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Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His>
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TAC CAG TCC AAG CCC CTG CTG TCA GCC TTC GCC GAC ATC ATC CAC TCA
Tyr Gln Ser Lys Pro Leu Leu Ser Ala Phe Ala Asp Ile Ile His Ser>
___a___a___a___a_TRANSLATION OF PRAE-7 MV [A] __a___a___a___a___>
820 830 840 850 860
CTA AAG GAG TCA GGG CAG CTC TGG CTA GAT GCC TAT CTT CAC CAA TAA
Leu Lys Glu Ser Gly Gln Leu Trp Leu Asp Ala Tyr Leu His Gln ***>
___a___a___a___a_TRANSLATION OF PRAE-7 MV [A] __a___a___a___a___>

Figure 15b

FastA Match of the Gene in pRAE-7 and the *M. alpina* Δ5-desaturase (Ma29) Translated Sequences

```
SCORES      Init1:    62 Initn:   105 Opt:   245
Smith-Waterman score: 271;    28.4% identity in 292 aa overlap

                                        10        20        30        40
pRAE-7.pep                      LLEPVSIGGIPAVQAQAGWLQ-HDFGHLSV-FSTSKWNHL--LH
                                                 ||:|    || :|:||  : : |: |   |
Ma29.pep        ASYYAQLFVPFVVERTWLQVVFAIIMGFACAQVGLNPLHDASHFSVTHNPTVWKILGATH
                     140       150       160       170       180       190

                          50        60        70        80        90
pRAE-7.pep      HFVIGHLKGAPASWWNHMH-FQHHAKPNCFRKDPDINM-HPFFFALGKILSVELGKQKKK
                  |    ::||    | ::| : ||   |    |||::  :|         :|:  | ::|
Ma29.pep        DF----FNGASYLVWMYQHMLGHHPYTNIAGADPDVSTSEP---------DVRRIKPNQK
                          200       210       220                 230

                100       110       120       130       140       149
pRAE-7.pep      YMPYNH--QHKYF-FLIGPPALLPLYFQWYIFYFVIQ----RKKWVDLAWMITFY--VRF
                ::  ||  || :  || |  |:     :  |:|||      | : ::    : |:    |
Ma29.pep        WF-VNHINQHMFVPFLYGLLAFKVRIQDINILYFVKTNDAIRVNPISTWHTVMFWGGKAF
              240       250       260       270       280       290

              150       160       170       180       190
pRAE-7.pep    FLTY---VPL--LGLKAFLGLFFIVRFLESNWFVWVTQMNHIPMHID---HDRN----MD
              |: |   |||  | |   | || :: :: | |:: : | ||:  :::    |:|     |
Ma29.pep      FVWYRLIVPLQYLPLGKVLLLFTVADMVSSYWLALTFQANHVVEEVQWPLPDENGIIQKD
            300       310       320       330       340       350

              200       210       220       230       240       250
pRAE-7.pep    WVSTQLQATCN-VHKSAFNDWFSGHLNFQIEHHLFPTMPRHNYHKVAPLVQSLCAKHGIE
              |:: |:::| : :| | :   ::| ||:|  |||||:: :|:|  :  :::: |::: :
Ma29.pep      WAAMQVETTQDYAHDSHLWTSITGSLNYQAVHHLFPNVSQHHYPDILAIIKNTCSEYKVP
            360       370       380       390       400       410

              260       270       280
pRAE-7.pep    YQSKPLL-SAFADIIHSLKESGQLWLDAYLHQX
              |  |  : :|||: :: |:  |
Ma29.pep      YLVKDTFWQAFASHLEHLRVLGLRPKEEX
            420       430       440
```

Figure 16

FastA Match of the Gene in pRAE-7 and *M. alpina* Δ6-desaturase (Ma524) Translated Sequences

```
SCORES      Init1:   278 Initn:   483 Opt:   301
Smith-Waterman score: 498;    31.9% identity in 285 aa overlap

                                    10        20        30        40
pRAE-7.pep                      LLEPVSIGGIPAVQAQAGWLQHDFGHLSVFSTSKWNHLLHHFVIG
                                                                    
Ma524.pep     GLSTVIVAKWGQTSTLANVLSAALLGLFWQQCGWLAHDFLHHQVFQDRFWGDLFGAFLGG
                140       150       160       170       180       190

                 50        60        70        80        90
pRAE-7.pep    HLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFF----FALGKILSV---ELGKQKKK
                                                                    
Ma524.pep     VCQGFSSSWWKDKHNTHHAAPNVHGEDPDIDTHPLLTWSEHALEMFSDVPDEELTRMWSR
                200       210       220       230       240       250

              100       110       120       130                   140
pRAE-7.pep    YMPYNHQHKYFFLIGPPALLPLYFQWYIFYFV-------------IQRKKWVDLAWMITF
                                                                    
Ma524.pep     FMVLN-QTWFYFPILSFARLSWCLQSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWIW
                260       270       280       290       300       310

                150       160       170       180       190       200
pRAE-7.pep    YVRFFLTYV--PLLGLKAFLGLFFIVRFLESNWFVWVTQMNHIPMHI---DHDRNMDWVS
                                                                    
Ma524.pep     YLATMFLFIKDPV----NMLVYFLVSQAVCGNLLAIVFSLNHNGMPVISKEEAVDMDFFT
                320       330       340       350       360       370

                      210       220       230       240       250       260
pRAE-7.pep    TQLQATCNVHKSAFNDWFSGHLNFQIEHHLFPTMPRHNYHKVAPLVQSLCAKHGIEYQSK
                                                                    
Ma524.pep     KQIITGRDVHPGLFANWFTGGLNYQIEHHLFPSMPRFNFSKIQPAVETLCKKYNVRYHTT
                      380       390       400       410       420       430

                      270       280
pRAE-7.pep    PLLSAFADIIHSLKESGQLWLDAYLHQX
                                    
Ma524.pep     GMIEGTAEVFSRLNEVSKAASKMGKAQX
                      440       450
```

Figure 17

FastA Match of the Gene in pRAE-7 and contig 2535

```
SCORES      Initl:  1028 Initn:  1424 Opt:  1430
Smith-Waterman score: 1430;    71.0% identity in 276 aa overlap

                              10        20        30        40        50
pRAE-7.pep                LLEPVSIGGIPAVQAQAGWLQHDFGHLSVFSTSKWNHLLHHFVIGHLKGAPA
                                        ||||||||||:|||||:   |||||:|:|||||||||| |
2535              VFYFGNGWIPTLITAFVLATSQAQAGWLQHDYGHLSVYRKPKWNHLVHKFVIGHLKGASA
                        10        20        30        40        50        60

                      60        70        80        90       100       110
pRAE-7.pep        SWWNHMHFQHHAKPNCFRKDPDINM-HPFFFALGKILSVELGKQKKKYMPYNHQHKYFFL
                  :|||| |||||||||| |:||||:|| |   |:||:   :| ||:| ||:||||||:||||
2535              NWWNHRHFQHHAKPNIFHKDPDVNMLH--VFVLGEWQPIEYGKKKLKYLPYNHQHEYFFL
                        70        80          90       100       110

                     120       130       140       150       160       170
pRAE-7.pep        IGPPALLPLYFQWYIFYFVIQRKKWVDLAWMITFYVRFFLTYVPLLG-LKAFLGLFFIVR
                  |||| |:|:|||: |:: :| :|:|||||| :::|:|||:||:|: | | |:| | || |
2535              IGPPLLIPMYFQYQIIMTMIVHKNWVDLAWAVSYYIRFFITYIPFYGILGALLFLNFI-R
                  120       130       140       150       160       170

                     180       190       200       210       220       230
pRAE-7.pep        FLESNWFVWVTQMNHIPMHIDHDRNMDWVSTQLQATCNVHKSAFNDWFSGHLNFQIEHHL
                  ||||:||||||||||| |:||::   || |:|| |||||::| ||||||||||||||||||
2535              FLESHWFVWVTQMNHIVMEIDQEAYRDWFSSQLTATCNVEQSFFNDWFSGHLNFQIEHHL
                  180       190       200       210       220       230

                     240       250       260       270       280
pRAE-7.pep        FPTMPRHNYHKVAPLVQSLCAKHGIEYQSKPLLSAFADIIHSLKESGQLWLDAYLHQX
                  |||||||| ||:||||:|||||||||||| |||| |: |||:|||:||:|||||||:|
2535              FPTMPRHNLHKIAPLVKSLCAKHGIEYQEKPLLRALLDIIRSLKKSGKLWLDAYLHKKSH
                  240       250       260       270       280       290

2535              SPRDTVGKGCRWGDGQRNDGLLFKGVSERLVYALLTDPMLDLSPFLLSFFSSHLPHSTLP
                  300       310       320       330       340       350
```

Figure 18

FastA Match of the Gene in pRAE-7 and contig 38

```
SCORES      Init1:   965 Initn:   965 Opt:   968
Smith-Waterman score: 968;    97.0% identity in 133 aa overlap

                                        10        20        30        39
pRAE-7.pep                          LLEPVSIGGIPAVQAQAGWLQHDFGHLSVFSTSKWNHLL
                                            :  ||||||||||||||||||||||||||||
38           LHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQHDFGHLSVFSTSKWNHLL
                130       140       150       160       170       180

            40        50        60        70        80        90        99
pRAE-7.pep   HHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFFALGKILSVELGKQKKKY
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
38           HHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFFALGKILSVELGKQKKKY
                190       200       210       220       230       240

           100       110       120       130       140       150       159
pRAE-7.pep   MPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWMITFYVRFFLTYVPLLGL
             |||||||  ||||||||||||||||||||||||||||||:
38           MPYNHQHXYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWISKQEYDEAGLPLSTANA
                250       260       270       280       290       300

           160       170       180       190       200       210       219
pRAE-7.pep   KAFLGLFFIVRFLESNWFVWVTQMNHIPMHIDHDRNMDWVSTQLQATCNVHKSAFNDWFS

38           SK
```

Figure 19

FastA Match of the N-terminus of Clone A-1 and Human Cytochrome b5

```
A-1.pdt
SW:CYB5_HUMAN

ID   CYB5_HUMAN     STANDARD;      PRT;   133 AA.
AC   P00167;
DT   21-JUL-1986 (REL. 01, CREATED)
DT   01-NOV-1988 (REL. 09, LAST SEQUENCE UPDATE)
DT   01-FEB-1996 (REL. 33, LAST ANNOTATION UPDATE)
DE   CYTOCHROME B5. . . .

SCORES      Init1:   127  Initn:   127  Opt:   183  z-score: 226.9 E(): 5.4e-06
Smith-Waterman score: 183;    32.2% identity in 90 aa overlap

                530        540        550        560        570        580
A-1.pdt      XLDLPTNMMEXRKAAAELXAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISE
                                                :|:| :|: ::: : ||:: :|||::::
CYB5_HUMAN                                  AEQSDEAVKYYTLEEIQKHNHSKSTWLILHHKVYDLTK
                                                10         20         30

                590        600        610        620        630        640
A-1.pdt      FTRRHPGGSRVISHYAGQDATDPFVAFHINKGLVKKYMN-SLLIGELSPEQPSFEPTKNK
             | ::|||| :|: : || |||: |    ::::   : |: :::|||| |::   :|  ||
CYB5_HUMAN   FLEEHPGGEEVLREQAGGDATENFE--DVGHSTDAREMSKTFIIGELHPDD---RPKLNK
             40         50         60         70         80         90

                650        660        670        680        690        700
A-1.pdt      ELTDEFRELRATVEQRFPVXFLTCTGAHGFFSLEVPGLPDSNKXFSWTSRPIXWNKGKRP

CYB5_HUMAN   PPETLITTIDSSSSWWINWVIPAISAVAVALMYRLYMAED
                100        110        120        130
```

Figure 20

FastA Match of 5' Sequence of Clone A-1 and ac004228

```
LOCUS       AC004228   170743 bp    DNA             HTG       26-FEB-1998
DEFINITION  *** SEQUENCING IN PROGRESS *** Homo sapiens Chromosome 11q12 pac
            pDJ519o3; HTGS phase 1, 18 unordered pieces.
ACCESSION   AC004228
NID         g2911733
KEYWORDS    HTG; HTGS_PHASE1. . . .


SCORES      Init1:   913 Initn:  1123 Opt:   916
  94.6% identity in 203 bp overlap

            389       379       369       359       349       339       330
A-1           CCCGACCAATATGATGGAATAAAGGAAAGCGGCCGCTGAATTATAGGCCGCCGAGACCGC
                                                    |||| | ||  ||||||||||||||||
ac004228      CCCGGCGCGCGGCGTCGCCAGGCCAGCTATGGCCCCCGACCCGGTGGCCGCCGAGACCGC
            60090     60100     60110     60120     60130     60140

            329       319       309       299       289       279       270
A-1           GGCTCAGGGACCTACCCCGCGTTACTTCACATGGGACGAGGTGGCCCAGCGCTCAGGGTG
              |||||||||||||||||||| ||||||||| ||||||||||||||||||||||||||||||
ac004228      GGCTCAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCCAGCGCTCAGGGTG
            60150     60160     60170     60180     60190     60200

            269       259       249       239       229       219       210
A-1           CGAGGAGCGGTGGCTTGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTCACCCGCCG
              ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
ac004228      CGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTCACCCGCCG
            60210     60220     60230     60240     60250     60260

            209       199       189       179       169       159       150
A-1           GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCCTT
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228      GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGTGAGCGC
            60270     60280     60290     60300     60310     60320

            149       139       129       119       109        99        90
A-1           CGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAACTCCTCCTGATTGG

ac004228      AGCCAGGCGGGGGCACAGGAGAGGGCGGGACCGGAGGCTGAGTGCAGGGGAGACAGAGTT
            60330     60340     60350     60360     60370     60380
```

Figure 21

FastA Match of 5' Sequence of Clone 3-5 and ac004228

SCORES Init1: 1365 Initn: 2510 Opt: 1377
98.6% identity in 285 bp overlap

```
                20        30        40        50        60        70
3-5             AATACGACTCACTATAGGGCTCGAGCGGCCGCCCCGGGCAGGTCCGGACCTGCCAACGTGA
                                                 ||||||| | ||||||||||||||||||
ac004228        CCCCGCCCCACACGCCGCATCACTTACAGGGCCCGGGGCTG-CCGGACCTGCCAACGTGA
                  61710     61720     61730     61740     61750     61760

                80        90        100       110       120       130
3-5             ATCTTATCGCCATGGACCTTACCTTGCACAACCCAAAGTAGCTGCCTTGGGGCAGGGGGT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228        ATCTTATCGCCATGGACCTTACCTTGCACAACCCAAAGTAGCTGCCTTGGGGCAGGGGGT
                  61770     61780     61790     61800     61810     61820

                140       150       160       170       180       190
3-5             GGCCAGAGTGCTTAGGGAAATGTGGAGCCCTACCCAGAACAACGGTGGAGGGAAAGGGAA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228        GGCCAGAGTGCTTAGGGAAATGTGGAGCCCTACCCAGAACAACGGTGGAGGGAAAGGGAA
                  61830     61840     61850     61860     61870     61880

                200       210       220       230       240       250
3-5             GAAACGCAGAAGTGCCCCAGTTCGGACGTAGGGAAGTCTTCCTCTTCGTGGTTTTTGGAG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228        GAAACGCAGAAGTGCCCCAGTTCGGACGTAGGGAAGTCTTCCTCTTCGTGGTTTTTGGAG
                  61890     61900     61910     61920     61930     61940

                260       270       280       290       300       310
3-5             AACCCTAGCTAAGAGAGGAAAGGGACTTATTGAAAGACCCGCAAGAAGGGACGGAAGTCT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228        AACCCTAGCTAAGAGAGGAAAGGGACTTATTGAAAGACCCGCAAGAAGGGACGGAAGTCT
                  61950     61960     61970     61980     61990     62000

                320       330       340       350       360       370
3-5             CATAGCCCTGAGAGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGT
                ||||||||||||||
ac004228        CATAGCCCTGAGAGGTGAAGCCAGCTGGAGTTGATGGGTCGAATGGGGACCTAGAGAACT
                  62010     62020     62030     62040     62050     62060
```

Figure 22

FastA Match of 5' Sequence of Clone A-10 and ac004228

```
SCORES      Init1:   931 Initn:  1309 Opt:   934
 97.0% identity in 200 bp overlap

           30        40        50        60        70        80        89
A-10        TATAGGGCTCGAGCGGCCGCCCGGGCAGGTGCCCGGAGGCGCCTGATCATACCTGTTGCC
                                                                        
ac004228    CGAGCCAAACACCGACTAATTCGGAGGAAAGCCCGGAGGCGCCTGATCATACCTGTTGCC
            60400     60410     60420     60430     60440     60450

           90       100       110       120       130       140       149
A-10        CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGGAGAGAGGCCTGGAAAAGTG
            |||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
ac004228    CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGGAGAGAGGCCTGGAGAAGTG
            60460     60470     60480     60490     60500     60510

          150       160       170       180       190       200       209
A-10        GAGTCTGGGGAGTGGGGATGGAGGCCAACAACACGCACACACAAACAAAGGGTCCCGCCT
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228    GAGTCTGGGGAGTGGGGATGGAGGCCAACAACACGCACACACAAACAAAGGGTCCCGCCT
            60520     60530     60540     60550     60560     60570

          210       220       230       240       250       260       269
A-10        CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGGATCCCTTTGTGGCCTTCCACAT
            ||||||||||||||||||||||||||||||||||| || ||  | ||
ac004228    CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGG-TCTCTGGGCGGGGACAGAACC
            60580     60590     60600     60610     60620     60630
```

Figure 23

FastA Match of 5' Sequence of Clone A-16 and ac004228

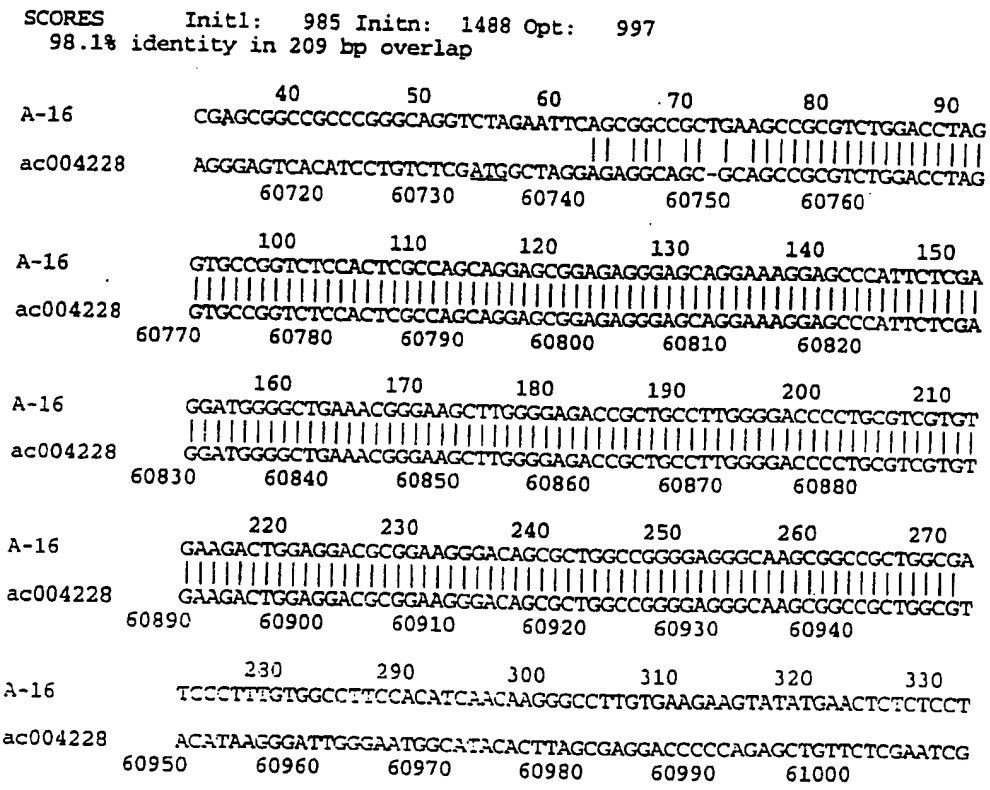

Figure 24

FastA Match of 5' Sequence of Clone A-19 and ac004228

```
SCORES      Init1:  1227 Initn:  1409 Opt:  1532
  94.0% identity in 349 bp overlap

                60        70        80        90       100       110
A-19            TTATTCCCTTATTTGTCCCTGCCCATGTCCTGCTGATTGGTCCATTTTACCTCTAGCTAG
                                                  ||||||||||||||||||||||||||||||
ac004228        ATAGAGCACTGATTGGTCCATTTTACAGGGTGCTGATTGGTCCATTTTACCTCTAGCTAG
             63250     63260     63270     63280     63290     63300

               120       130       140       150       160       170
A-19            CTAAAGAGCACGGATTGGTGCATTTTGCAAACCTCTGGCTACAGAGGGGTTCTCCAGGTC
                |||||||||||||||||||||||| ||||||||| ||||||||  |||||||| |||
ac004228        CTAAAGAGCACGGATTGGTGCATTTTACAAACCTCTAGCTACAGAAAAGTTCTCCAAGTC
             63310     63320     63330     63340     63350     63360

               180       190       200       210       220       230
A-19            TGCACTCGACCCAGGAAGTCCATCTGGCTTCACCTCTCACTTCAACTTGGGTACAGCCTT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ac004228        TGCACTCGACCCAGGAAGTCCATCTGGCTTCACCTCTCACTTCAACTTGGGTACAGCCTT
             63370     63380     63390     63400     63410     63420

               240       250       260       270       280       290
A-19            CTGGCGGGCAGGAAGATGGCCTTTGGTGCGAACACTGCCGGAGTCCAGGGGGCTGGCTCC
                |||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||
ac004228        CTGGCGGGCAGGAGGATGGCCTTTGGTGCGAACACTGCCGGAGTCCAGGGGGCTGGCTCC
             63430     63440     63450     63460     63470     63480

                300       310       320       330       340
A-19            CTCACCTTTCATCTTCTCCCGGCACTTGCAGGATCCCTTTGTGGCC
                |||||||||||||||||||||||||||||||||||||||||||||
ac004228        CTCACCTTTCATCTTCTCCCGGCACTTGCAGGATCCCTTTGTGGCC
             63490     63500     63510     63520     63530
```

Figure 25

Partial Sequence of ac004228

59751 ACTAGAACCG CTGTTCCTAC CGCGGCGCCC CCTGGGAGCC AACGCCGCGA

59801 TGCCCGCCTG ACGTCAGGAA GTCGAATCCG GCGGCGACGC TTTTAGGGAG

59851 CCCGCGAGGG GGCGCGTGTT GGCAGCCCAG CTGTGAGTTG CCCAAGACCC

59901 ACCGGGGGAC GGGATCTCGC TCCCCGCGCC ACGAGGCTCG GCCAATGGGA Possible start

59951 ACGCGCGCTG CGAGGCCCGC CGGTCTGCCC TGCGGTGCTG AAAACCCGGC

60001 GCGCAGGCGG CTGGCTCTGG GCGCGCGCCA GCAAATCCAC TCCTGGAGCC

60051 CGCGGACCCC GAGCACGCGC CTGACAGCCC CTGCTGGCCC GGCGCGCGGC

60101 GTCGCCAGGC CAGCTATGGC CCCCGACCCG GTGGCCGCCG AGACCGCGGC

60151 TCAGGGACCT ACCCCGCGCT ACTTCACCTG GGACGAGGTG GCCCAGCGCT Clone A-1

60201 CAGGGTGCGA GGAGCGGTGG CTAGTGATCG ACCGTAAGGT GTACAACATC

60251 AGCGAGTTCA CCCGCCGGCA TCCAGGGGGC TCCCGGGTCA TCAGCCACTA

60301 CGCCGGGCAG GATGCCACGG TGAGCGCAGC CAGGCGGGGG CACAGGAGAG

60351 GGCGGGACCG GAGGCTGAGT GCAGGGGAGA CAGAGTTACG CACTCCGAGC

60401 CAAACACCGA CTAATTCGGA GGAAAGCCCG GAGGCGCCTG ATCATACCTG

60451 TTGCCCGGTG ATTGGGTGTC CTGCGGATGC GGGATGAAAA GGCGGGAGAG Clone A-10

60501 AGGCCTGGAA AAGTGGATTC TGGGGAGTGG GGATGGAGGC CAACAACACG

60551 CACACAAAAA CAAAGGTTCC TGCCTCCCTG CCGTGCATTC CATCTGCAGC

60601 CCCGAGCCTC AGGTCTCCTG GCGGGGACAG AACCCCGAGC TGGGTAGGCT

60651 AGGAGGGAGG AGAGCAAAGA TGCAGGCCGC CTGGGGAGGG AGGGGGTCAG

60701 TGGCCAAGGG AGGGAGTCAC ATCCTGTCTC GATGGCTAGG AGAGGCAGCG

60751 CAAGGGGTTC TGGACCTAGG TGCCGGTCTC CACTCGCCAG CAGGAGCGGA Clone B-17

60801 GAAGGAGCAG GAAAGGAGCC CATTCTCGAG GATGGGGCTG AAACGGGAAG

60851 CTTCGGGAAA CGCCTGGTTT GGGGACCCCT GCGTCGTGTG AAGACTGGAG

60901 GACGCGGAAG GGACAGCGCT GGCCGGGGAG GGCAAGCGGC CGCTGGCGTA

60951 CATAAGGGAT TGGGAATGGC ATACACTTAG CGAGGACCCC CAGAGCTGTT

61001 CTCGAATGGC CGGGGAGGCC AGTGAGCCGC AGGCCAGCGA GGTCTTCAGC

Figure 26a

61051 TATTCCGCGG AGCGGACCGC TGTTTACGCT CTGGGGCGGT AGGCCCTTCG

61101 CGGGGTCCTG TCCCTTCTTC CCTTGGTCTC ACTGCGGGGT CGGCGCGCGC

61151 CCCAGCCCCA GGCCTGCTGC TTCCCTTTCT AGACCACAGC CCTCAGAGCT

61201 AAGGCCCCGG CGCCTCTCTG CTGGGTTGGA GTCCTGGGGA CTCAGTCCTA

61251 GGGACTCGAA AGTCGGGGCG TTCCCTTCAC CGCGTTTCCC CCTTGGCGGC

61301 CAGAATGGCG TCCCCTCCCC TTGCATCCCC CTCTGATCCC GTGCCCTGCA

61351 GCGTGATGCC CTCCACTGTC CCTATCCACT ACCCTGGCGT CCCAGAGTGT

61401 GCCGCGGGTC ACCAGGTTCC CATAACGTCG CAGCAGAGCT TAGACGCTGC

61451 GGGGCGAAGA CCCGCCCCAC CCTCTGACGC GACCAGCCTA GTGGGCGAGG

61501 CCAGAGCTTG CGCGGGTCAA CCAGAGTGAC CACTCGGGAG CCCTGACTGC

61551 GGCCAAGGGC GCAGGCGTGT CCCGGCGCAT GCGCAGACGA AACAGGCACC

61601 AACGCTGGAG CTTCCCGCAG TGTGATTTGG GGCCGGGGAT GCCGCGGCGG

61651 GGACGGCGAT TGGTCCGTAT GTGTGGTGCC ACCGGCCGCC GCTCCGCCCC

61701 GGCCCCCGCC CCACACGCCG CATCACTTAC AGGGCCCGGG GCTGCCGGAC

61751 CTGCCAACGT GAATCTTATC GCCATGGACC TTACCTTGCA GAACCCAAAG

61801 TAGCTGCCTT GGGGCAGGGG GTGGCCAGAG TGCTTAGGGA AATGTGGAGC Clone 3-5

61851 CCTACCCAGA ACAACGGTGG AGGGAAAGGG AAGAAACGCA GAAGTGCCCC

61901 AGTTCGGACG TAGGGAAGTC TTCCTCTTCG TGGTTTTTGG AGAACCCTAG

61951 CTAAGAGAGG AAAGGGACTT ATTGAAAGAC CCGCAAGAAG GGACGGAAGT

62001 CTCATAGCCC TGAGAGGTGA AGCCAGCTGG AGTTGATGGG TCGAATGGGG

62051 ACCTAGAGAA CTTTTCTGTA TCTAGAGGTT TGTAAAATGC ACCAATCAGT

62101 GCTCTGTAAA AACGCACCAA TTGGCGCTCT GTAGCTAGCT AGAGGTTTGT

62151 AAAATGAGCC AATCAGCAGG ACGTGGGCAG GGACAACTAA GACAATAAAA

62201 GCTGGCCACC CCAGCCAGCT GCTGCAACCC GCTCCAGTTC CCTTACAGGC

62251 TGTGGAAGCA TTGTTCTTTT GCTCGTCACA CTAAACCTTG CTGCTGCTCA

62301 TTCTTTGGGT CTGCAAAGAG TGTTATTCCT TTAAGAGCTA TAACAGCGGG

62351 AAGGTCCACG GCTCCATTCT TGAAGTCAGT GAGACCATAC CCGCCGGAAG

62401 GAACCAACGC CCGACACAGC CCCACCCATC TCTCCTGTTT CTCACCTATA

Figure 26b

62451 CTGAAATTCT TGGGCAAAAG CTGTCTGTGG ACACACCCAG GGGAAAGGCC

62501 AGCCCAGGCA GGTGTTTCTT AGTGGTTCCC CTCAGCCAAT GCTTCCCATT

62551 CCTTGATGCA TCCTTCTAAC TAGAGCAGAT GCTCGGTGAT CTTAAACTGT

62601 GGACACCTGG GAGCACCCTC AAAAGGCAGC TGGGCCTAGG GAGATGGCCT

62651 GTGCTTCTGT GTCAGGAGTT GGTTCCTTCA GGTGGGCTTG TGGTCTCGCT

62701 GACGTCAAGA ATGAAGCCAT GAACCTTCGC GGTGAGTGTT ACAGCTCTTA

62751 CAGGTGGCGT GGACCCAAAG AGTGAGCAGC AGCAAGATTT ATTGTGAAGA

62801 GCAAAGAACA AAGCTTCCAC AGCGTGGAAG GGTACCCGAG CAGGTTGCCG

62851 CTGCTGGACG TTGGGGGGTG TGAGGGGGAG CAGCCTTTTT TTTTCTTTTT

62901 TTTTTGAGAC GGAGTCTCCC TGTCGCCCAG GCTGGAGTGC AGTGGCGCGA

62951 TCTCGGCTCA CTGCAGGCTC CGCCCCCCCC CCGGGGTTCA CGCCATTCTC

63001 CTGCCTCAGC CTCCCGAGTA GCTGGGACTA CAGGCGCCCG CTACCTCGCC

63051 CGGCTAATTT TTTGTATTTT TAGTAGAGAC GGGGTTTCAC TGTGTTAGCC

63101 AGGATGGTCT CGATCTCCTG ACCTCGTGAT CCACCCGCCT TGGCCTCCCA

63151 AAGTGCTGGG ATTACAGGCG TGAGCCACCG CGCCCGGCCG GGAGCAGCTT

63201 TTATTCCCTT ATTTGTCCCT GCCCATGTCC TGCTGATTTG TCCATTTTAT

63251 AGAGCACTGA TTGGTCCATT TTACAGGGTG CTGATTGGTC CATTTTACCT

63301 CTAGCTAGCT AAAGAGCACG GATTGGTGCA TTTTACAAAC CTCTAGCTAC

63351 AGAAAAGTTC TCCAAGTCTG CACTCGACCC AGGAAGTCCA TCTGGCTTCA

63401 CCTCTCACTT CAACTTGGGT ACAGCCTTCT GGCGGGCAGG AGGATGGCCT

63451 TTGGTGCGAA CACTGCCGGA GTCCAGGGGG CTGGCTCCCT CACCTTTCAT Clone B-19

63501 CTTCTCCCGG CACTTGCAGG ATCCCTTTGT GGCCTTCCAC ATCAACAAGG

63551 GCCTTTGTGAA GAAGTATATG AACTCTCCCC TGATTGGAGA ACTGTCTCCA

63601 GAGCAGCCCA GCTTTGAGCC CACCAAGAAT GTAAGACCCT GTGTTTGCTA

63651 TGTCCCAACT ATTGGTTGTT GAGGGGGACA GAGAGGGGGT GGAAGGAGAG

63701 TCTAGATGGA ATCACAGTCA TAGTAATCAC AGTCAGTAGT AGCTCTGGGG

63751 AGTCTTGAGG TCCCTGCTTC TCTTGCATAG TCATGAGGTC ACAGGCCCAA

Figure 26c

63801 GGGAGCATGG CTTTGCAACC TATGGCTCCC CCAAGGCTGC CACTACCATG

63851 GCTGCCATCA TTGTTATCAT CATTGTTATC ATATGAGCAC TTACTATGCA

63901 CCAAGCATAA ACTCATAACT CTTACACATT TACAGATGAG ATAACAGGCT

63951 CAGGGAGGTT AAGCAACACA GCCAAGGATC ACACAGTTAG TAAATGGCAG

64001 AGCAAGGACT TAGTCCCCTG AACTCTTAGG CACTATCCCA TGGCACCTCC

64051 TCCTGTCATC CTCATTGTCG TGGTATCTTT GCCTAGGACT GTGGACTTCC

64101 CACAGCTACC TCAGTGGGAG GTCCTTGAGC CTGAGAGGGC CCTTGTCTCC

64151 AGTAGCATTG GGGTGCAGAT GAGAAGAATA ACAGCTCCTC TTCCTCTTCT

64201 GCAGAAAGAG CTGACAGATG AGTTCCGGGA GCTGCGGGCC ACAGTGGAGC

64251 GGATGGGGCT CATGAAGGCC AACCATGTCT TCTTCCTGCT GTACCTGCTG

64301 CACATCTTGC TGCTGGATGG TGCAGCCTGG CTCACCCTTT GGGTCTTTGG

64351 GACGTCCTTT TTGCCCTTCC TCCTCTGTGC GGTGCTGCTC AGTGCAGTTC

64401 AGGTGAGAGC CTTTGGCTTG TCAAGTGCAC AGCAATGCTC AGCATCCCTG

Figure 26d

FastA Match of Human Δ5-desaturase and Contig 3381584

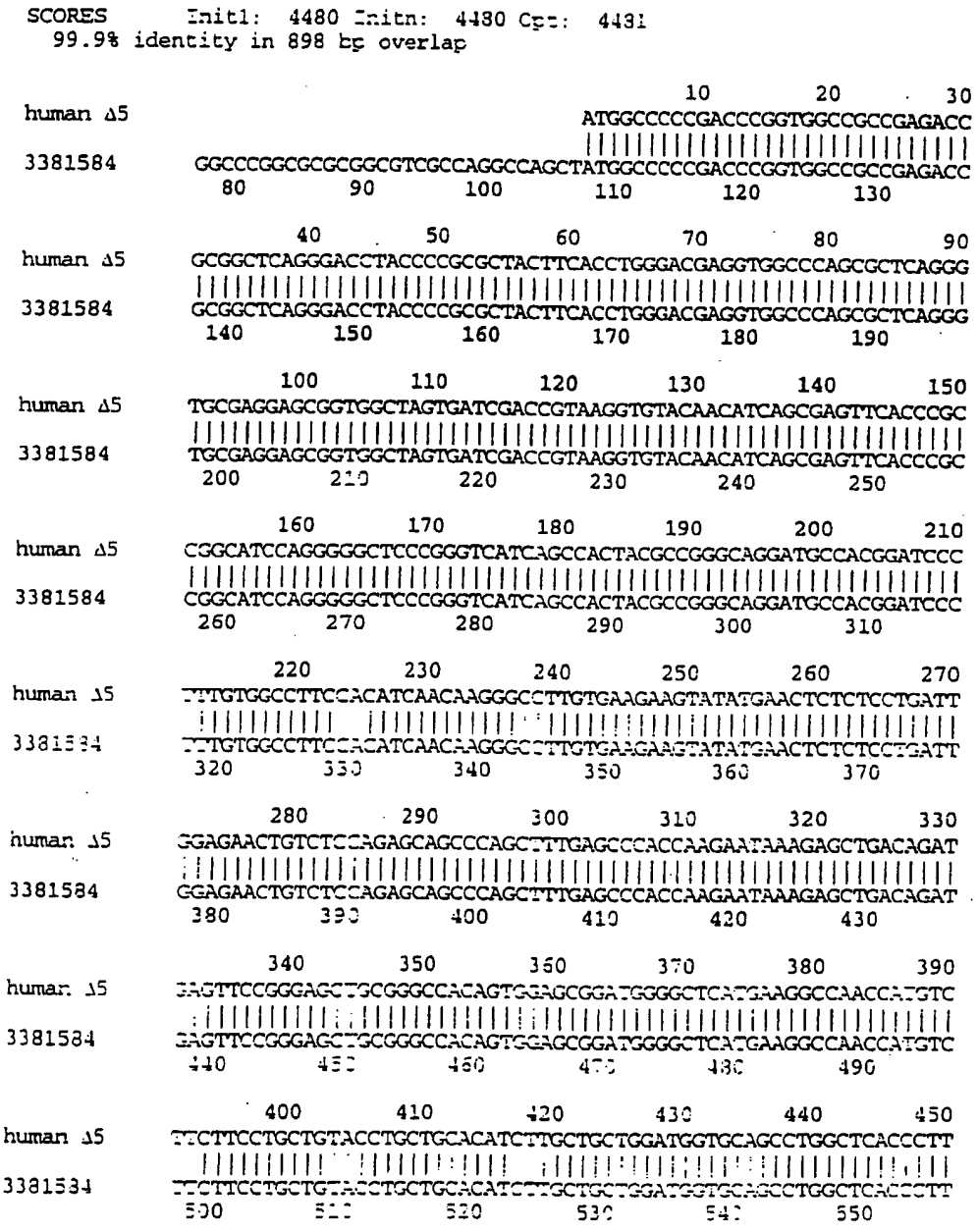

Figure 27a

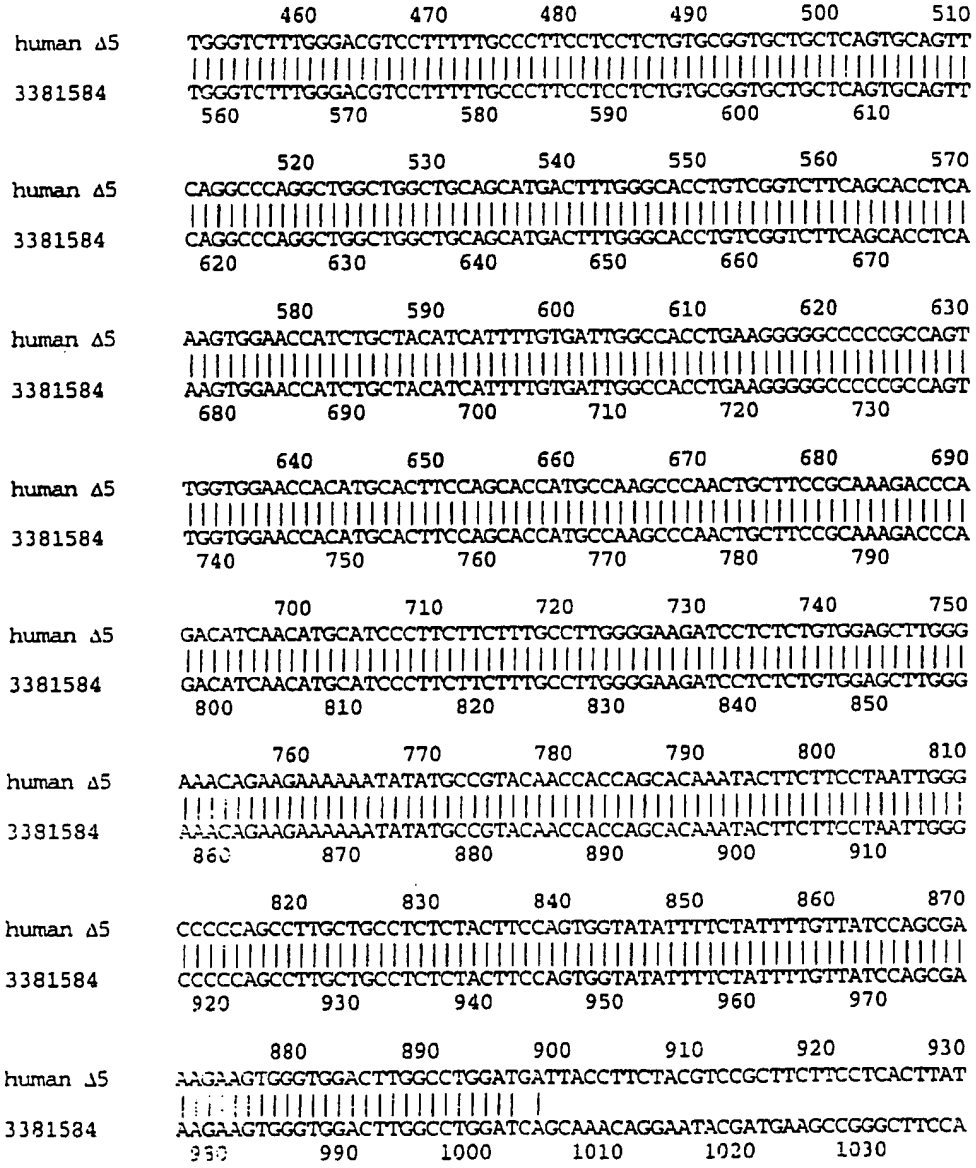
460 470 480 490 500 510
human Δ5 TGGGTCTTTGGGACGTCCTTTTTGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTT
3381584 TGGGTCTTTGGGACGTCCTTTTTGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTT
560 570 580 590 600 610
520 530 540 550 560 570
human Δ5 CAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA
3381584 CAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA
620 630 640 650 660 670
580 590 600 610 620 630
human Δ5 AAGTGGAACCATCTGCTACATCATTTTGTGATTGGCCACCTGAAGGGGGCCCCCGCCAGT
3381584 AAGTGGAACCATCTGCTACATCATTTTGTGATTGGCCACCTGAAGGGGGCCCCCGCCAGT
680 690 700 710 720 730
640 650 660 670 680 690
human Δ5 TGGTGGAACCACATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA
3381584 TGGTGGAACCACATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA
740 750 760 770 780 790
700 710 720 730 740 750
human Δ5 GACATCAACATGCATCCCTTCTTCTTTGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG
3381584 GACATCAACATGCATCCCTTCTTCTTTGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG
800 810 820 830 840 850
760 770 780 790 800 810
human Δ5 AAACAGAAGAAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG
3381584 AAACAGAAGAAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG
860 870 880 890 900 910
820 830 840 850 860 870
human Δ5 CCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTTCTATTTTGTTATCCAGCGA
3381584 CCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTTCTATTTTGTTATCCAGCGA
920 930 940 950 960 970
880 890 900 910 920 930
human Δ5 AAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTCACTTAT
3381584 AAGAAGTGGGTGGACTTGGCCTGGATCAGCAAACAGGAATACGATGAAGCCGGGCTTCCA
990 1000 1010 1020 1030

Figure 27b

FastA Match of Human Δ5-desaturase and Contig 2153526

SCORES Init1: 1892 Initn: 1892 Opt: 2161
98.6% identity in 443 bp overlap

```
                870       880       890       900       910       920
human Δ5        TCCAGCGAAAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCC
                                                  | ||::|||||||||||||||||||||||
2153526                                           GAATKMTTACCTTCTACGTCCGCTTCTTCC
                                                        10        20        30

                930       940       950       960       970       980
human Δ5        TCACTTATGTGCCACTATTGGGGCTGAAAGCCTTCCTGGGCCTTTTCTTCATAGTCAGGT
                |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
2153526         TCACTTATGTGCCACTATTGGGGCTGAAAG-CTTCCTGGGCCTTTTCTTCATAGTCAGGT
                        40        50        60        70        80

                990       1000      1010      1020      1030      1040
human Δ5        TCCTGGAAAGCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCCATGCACATTG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
2153526         TCCTGGAAAGCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCCATGCACATTG
              90        100       110       120       130       140

                1050      1060      1070      1080      1090      1100
human Δ5        ATCATGACCGGAACATGGACTGGGTTTCCACCCAGCTCCTGGCCACATGCAATGTCCACA
                |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
2153526         ATCATGACCGGAACATGGACTGGGTTTCCACCCAGCTCCAGGCCACATGCAATGTCCACA
            150       160       170       180       190       200

                1110      1120      1130      1140      1150      1160
human Δ5        AGTCTGCCTTCAATGACTGGTTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTT
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
2153526         AGTCTGCCTTCAATGACTGGTTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTT
            210       220       230       240       250       260

                1170      1180      1190      1200      1210      1220
human Δ5        TTCCCACGATGCCTCGACACAATTACCACAAAGTGGCTCCCCTGGTGCAGTCCTTGTGTG
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
2153526         TTCCCACGATGCCTCGACACAATTACCACAAAGTGGCTCCCCTGGTGCAGTCCTTGTGTG
            270       280       290       300       310       320

                1230      1240      1250      1260      1270      1280
human Δ5        CCAAGCGTGGCATAGAGTACCAGTCCAAGCCCCTGCTGTCAGCCTTCGCCGACATCATCC
                |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
2153526         CCAAGCATGGCATAGAGTACCAGTCCAAGCCCCTGCTGTCAGCCTTCGCCGACATCATCC
            330       340       350       360       370       380

                1290      1300      1310      1320      1330
human Δ5        ACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCTATCTTCACCAATAA
                |||||||||||||||||||||||||||||||||||||||||||||||||||
2153526         ACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCTATCTTCACCAATAACAACAGC
            390       400       410       420       430       440
```

Figure 28

FastA Match of Human Δ5-desaturase and Contig 253538a

```
SCORES      Init1:  1479 Initn:  2483 Opt:  2489
Smith-Waterman score: 2489;    81.3% identity in 434 aa overlap

                    10        20        30        40        50        60
human Δ5     MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS
                                                                        
253538a                  QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS
                                10        20        30        40

                   70        80        90       100       110       120
human Δ5     HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRATVE
                                                                        
253538a      HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRATVE
             50        60        70        80        90       100

                  130       140       150       160       170       180
human Δ5     RMGLMKANHVFFLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQHD
                                                                        
253538a      RMGLMKANHVFFLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQHD
            110       120       130       140       150       160

                  190       200       210       220       230       239
human Δ5     FGHLSVFSTSKWNHLLHHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINM-HPFFF
             :|||||:   |||||:|:|||||||| |:|||| |||||||| |:||||:|| |   |
253538a      YGHLSVYRKPKWNHLVHKFVIGHLKGASANWWNHRHFQHHAKPNIFHKDPDVNMLH--VF
            170       180       190       200       210       220

            240       250       260       270       280       290       299
human Δ5     ALGKILSVELGKQKKKYMPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWM
             :||:   :| ||:| ||:|||||:|||||||| |:|:|||: |:: :| :|:||||||
253538a      VLGEWQPIEYGKKGLKYLPYNHQHEYFFLIGPPLLIPMYFQYQIIMTMIVHKNWVDLAWA
              230       240       250       260       270       280

            300       310       320       330       340       350
human Δ5     ITFYVRFFLTYVPLLG-LKAFLGLFFIVRFLESNWFVWVTQMNHIPMHIDHDRNMDWVST
             :::|:|||:||:|: | | |:| | || |||||:|||||||||| |:||::   || |:
253538a      VSYYIRFFITYIPFYGILGALLFLNFI-RFLESHWFVWVTQMNHIVMEIDQEAYRDWFSS
              290       300       310       320       330       340

            360       370       380       390       400       410
human Δ5     QLLATCNVHKSAFNDWFSGHLNFQIEHHLFPTMPRHNLHKVAPLVQSLCAKRGIEYQSKP
             || |||||::| |||||||||||||||||||||||| ||:||||:|||||:||||| ||
253538a      QLTATCNVEQSFFNDWFSGHLNFQIEHHLFPTMPRHNLHKIAPLVKSLCAKHGIEYQEKP
               350       360       370       380       390       400

            420       430       440
human Δ5     LLSAFADIIHSLKESGQLWLDAYLHQX
             || |: |||:|||:||:||||||||:|
253538a      LLRALLDIIRSLKKSGKLWLDAYLHKKSHSPRDTVGKGCRWGDGQRNDGLLFKGVSERLV
               410       420       430       440       450       460
```

Figure 29

FastA Match of Human Δ5-desaturase and Contig 38

```
SCORES      Init1:  2024 Initn:  2024 Opt:  2026
Smith-Waterman score: 2026;    99.3% identity in 287 aa overlap

                    10        20        30        40        50        60
human Δ5     MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||
38                  QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS
                              10        20        30        40

                          70        80        90       100       110       120
human Δ5     HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRATVE
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
38           HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRATVE
                    50        60        70        80        90       100

                         130       140       150       160       170       180
human Δ5     RMGLMKANHVFFLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQHD
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
38           RMGLMKANHVFFLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQHD
                  110       120       130       140       150       160

                         190       200       210       220       230       240
human Δ5     FGHLSVFSTSKWNHLLHHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFFA
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
38           FGHLSVFSTSKWNHLLHHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFFA
                  170       180       190       200       210       220

                         250       260       270       280       290       300
human Δ5     LGKILSVELGKQKKKYMPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWMI
                    |||||||||||||||||||| ||||||||||||||||||||||||||||||||:
38           LGKILSVELGKQKKKYMPYNHQHXYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWIS
                  230       240       250       260       270       280

                         310       320       330       340       350       360
human Δ5     TFYVRFFLTYVPLLGLKAFLGLFFIVRFLESNWFVWVTQMNHIPMHIDHDRNMDWVSTQL

38           KQEYDEAGLPLSTANASKRDLPRATSPGTRWPSAQGARSGGXXSTVRCTTSASSPAGIQG
                  290       300       310       320       330       340
```

Figure 30

FastA Match of Δ6-Desaturase (Ma524) and Human Δ5-Desaturase

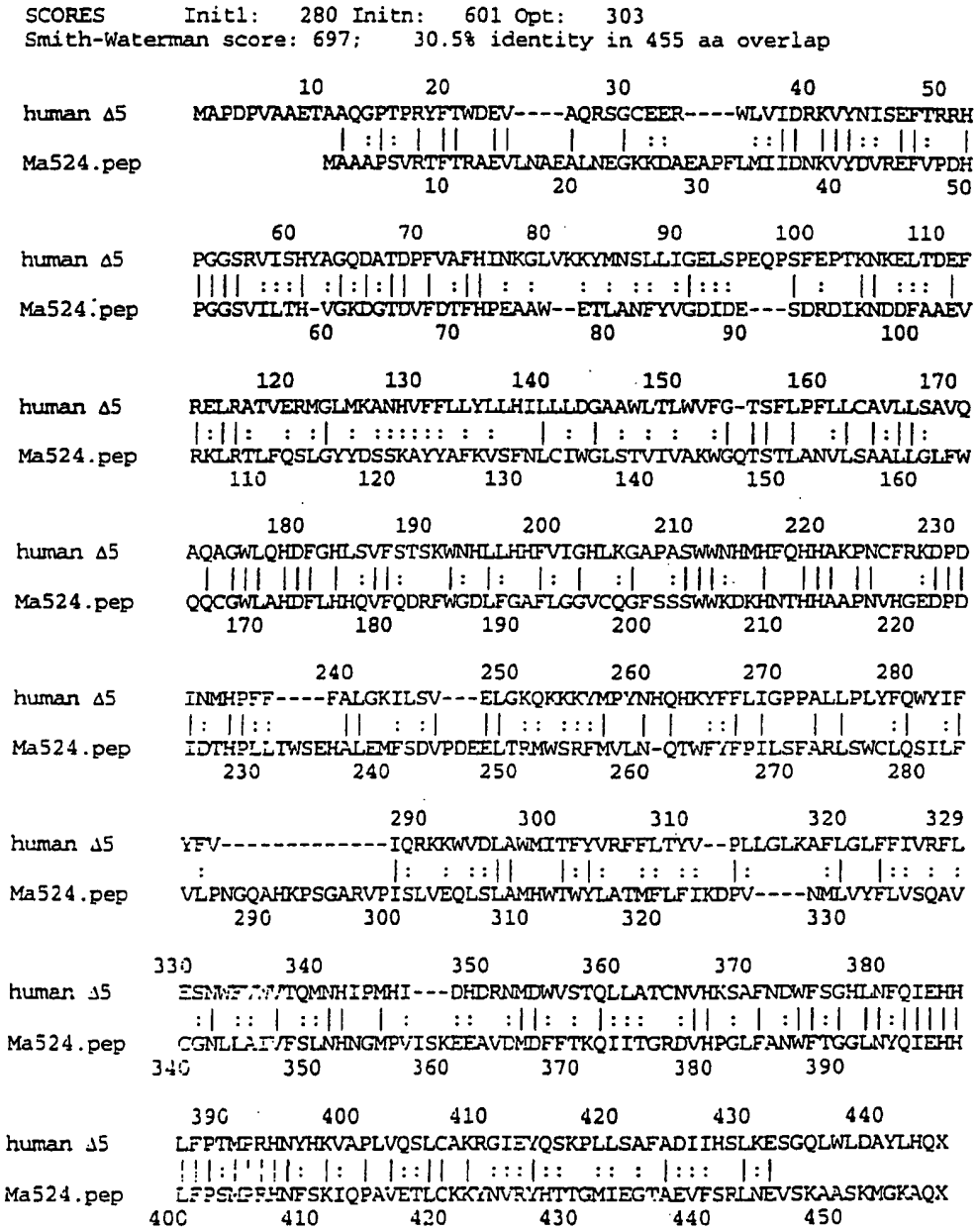

Figure 31

FastA Match of Δ5-Desaturase (Ma29) and Human Δ5-Desaturase

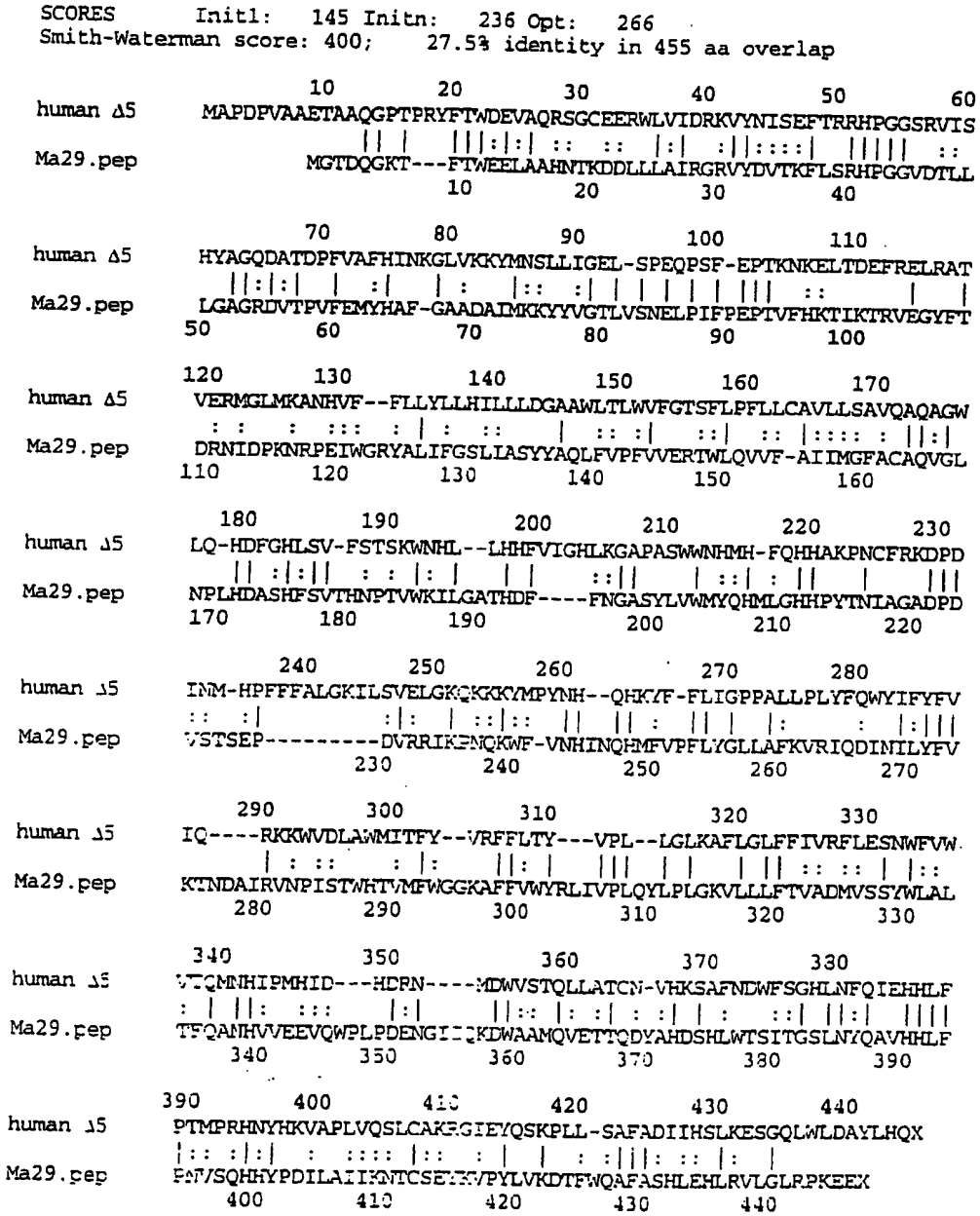

Figure 32

Figure 33

Host(plasmid)	334(pRAE-28-5)		334(pRAE-26-1)		334(pRAE-33)		334(pRAE-35)		334(pYX242)	
Added substrate	25 µM DGLA		25 µM DGLA		25 µM DGLA 30°C/48 hrs		25 µM DGLA		25 µM DGLA	
Fatty acid			(g fatty acid/100 g fatty acid)			lipid (µg)				
C16:0		151.580		202.175		285.291		281.298		304.229
C16:1		406.279		485.631		552.951		569.298		608.123
C18:0		16.494		25.995		32.162		27.479		30.093
C18:1n-9		100.031		133.349		173.772		184.740		187.780
C18:2n-6				0.180						0.946
C18:3n-6				0.058		0.074				0.074
C20:0		3.844		4.205		7.118		7.285		6.288
C20:3n-6		96.576		118.657		134.859		139.292		125.448
C20:4n-6	(0.127%)	1.204	(0.075%)	0.878	(0.062%)	0.902	(0.063%)	0.927	(0.062%)	0.958
C22:0				0.150		0.119				0.125
C22:1n-9		0.162		0.139		0.299		0.275		0.392
Total Lipid		949.0		1169.0		1445.5		1468.0		1538.5

Figure 34a

Host(plasmid)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)
Added substrate	25 µM DGLA	25 µM DGLA	25 µM DGLA 30°C/48 hrs	25 µM OA	25 µM OA	25 µM OA	25 µM AA	25 µM AA	25 µM AA 30°C/48 hrs
Fatty acid	(g fatty acid/100 g fatty acid)			lipid (µg)					
C16:0	49.332	106.358	93.225	84.327	37.013	51.018	78.471	53.685	74.099
C16:1	141.178	256.622	277.028	269.009	107.066	172.485	230.45	141.526	181.298
C18:0	9.301	14.819	12.908	11.871	8.3	9.047	11.283	9.97	10.969
C18:1n-9	39.876	87.564	72.842	106.416	52.634	71.453	61.754	42.289	46.873
C18:2n-6				ND	ND	ND			
C18:3n-6									
C20:0	2.154	7.339			3.729			2.685	
C20:3n-6	45.395	56.346	55.306						60.89
C20:4n-6	(0.106%) 0.412	(0.060%) 0.396	(0.065%) 0.402				(0.026%) 0.139	(0.019%) 0.077	(0.027%) 0.126
C20:5n-3							63.584	68.442	
Total Lipid	387	665	620	562	284	363	535	404	466

Figure 34b

Host(plasmid)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)
Added substrate	25 µM LA	25 µM LA	25 µM LA 30°C/48 hrs	no substrate	no substrate	no substrate
Fatty acid	(g fatty acid/100 g fatty acid)			lipid (µg)		
C16:0	56.631	45.393	74.247	174.138	25.574	33.44
C16:1	181.311	117.045	208.029	277.122	43.193	47.189
C18:0	9.549	9.251	11.45	22.547	5.119	8.432
C18:1n-9	48.256	46.496	51.342	134.822	21.89	32.618
C18:2n-6	31.91	23.221	36.821			
C18:3n-6	(0.02%) 0.082	ND	(0.012%) 0.056			
C20:0		0.339		0.702		
C20:3n-6						
C20:4n-6						
C20:5n-3	0.121					
Total Lipid	407	279	460	746	127	168

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(81) Designated States: AU, BG, BR, CA, CN, CZ, HU, IL, JP, KR, MX, NO, NZ, PL, RO, SI, SK, TR, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) Title: HUMAN DESATURASE GENE AND USES THEREOF

(57) Abstract

The subject invention relates to the identification of a gene involved in the desaturation of polyunsaturated fatty acids at carbon 5 (i.e., "human Δ5-desaturase") and to uses thereof. In particular, human Δ5-desaturase may be utilized, for example, in the conversion of dihomo-γ - linolenic acid (DGLA) to arachidonic acid (AA) and in the conversion of 20:4n-3 to eicosapentaenoic acid (EPA). AA or polyunsaturated fatty acids produced therefrom may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/US 99/31163

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N9/02 C12N15/64 C12N15/81 C12N15/82 C12P7/64
C12N5/16 A01K67/00 A23L1/00 A61K31/202 A61K7/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P A01K A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 46765 A (CALGENE LLC; ABBOTT LABORATORIES) 22 October 1998 (1998-10-22) SEQ ID NO:24 and SEQ ID NO:34 page 65, line 10 - line 19 & WO 98 46763 A 22 October 1998 (1998-10-22) & WO 98 46764 A ---	1,3, 6-29, 31-56
X	LAMERDIN J.E. ET AL.: "BC269730_2." EMBL DATABASE ENTRY 060427; ACCESSION NO. 060427, 1 August 1998 (1998-08-01), XP002140846 --- -/--	6,7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2000	12/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Schönwasser, D

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INTERNATIONAL SEARCH REPORT

Interi nal Application No
PCT/US 99/31163

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHO H. P. ET AL.: "Cloning, Expression, and Fatty Acid Regulation of the Human Delta-5 Desaturase" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 52, 24 December 1999 (1999-12-24), pages 37335-37339, XP002140847 ISSN: 0021-9258 figure 1 ---	1,3-22
E	WO 00 20603 A (ABBOTT LABORATORIES) 13 April 2000 (2000-04-13) SEQ ID NO:24 and SEQ ID NO:31 ---	1,3, 6-29, 31-56
A	MICHAELSON L ET AL: "Isolation of a delta5-fatty acid desaturase gene from Mortierella alpina" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 30, 24 July 1998 (1998-07-24), pages 19055-19059, XP002076636 ISSN: 0021-9258 the whole document ---	1-56
A	KNUTZON D S ET AL: "Identification of Delta5 - desaturase from Mortierella alpina by heterologous expression in Bakers yeast and canola" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 45, 6 November 1998 (1998-11-06), pages 29360-29366, XP002106760 ISSN: 0021-9258 the whole document -----	1-56

2

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-36,55,56

An isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence represented by SEQ ID NO:1; a purified protein encoded by said nucleotide sequence; a purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of said purified protein; a method of producing a human delta5-desaturase comprising inter alia the step of isolating said nucleotide sequence represented by SEQ ID NO:1; a vector comprising a) a nucleotide sequence as represented by SEQ ID NO:1 operably linked to b) a promoter; a host cell comprising said vector; a plant cell, plant, or plant tissue comprising said vector; one or more plant oils or acids expressed by said plant cell, plant, or plant tissue; a transgenic plant comprising said vector; a mammalian cell comprising said vector; a transgenic,non-human mammal whose genome comprises a DNA sequence encoding a human delta5-desaturase operably linked to a promoter; a fluid produced by said transgenic, non-human mammal and a method for producing a polyunsaturated fatty acid comprising inter alia the step of isolating said nucleotide sequence represented by SEQ ID NO:1.

2. Claims: 37-45,54

A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid and a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition in an amount sufficient to effect said treatment.

3. Claims: 46-48

A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid and 2) a pharmaceutically acceptable carrier.

4. Claims: 49-52

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid.

5. Claim : 53

A cosmetic comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to above mentioned method of producing a polyunsaturated fatty acid.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter. ...nal Application No
PCT/US 99/31163

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9846765 A	22-10-1998	US 5972664 A	26-10-1999
		AU 7114798 A	11-11-1998
		AU 7114898 A	11-11-1998
		EP 0996732 A	03-05-2000
		EP 1007691 A	14-06-2000
		NO 994924 A	30-11-1999
		NO 994926 A	30-11-1999
		WO 9846764 A	22-10-1998
		US 6051754 A	18-04-2000
WO 0020603 A	13-04-2000	NONE	